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THE PASSIVE PROTECTION OF MICE AGAINST INFECTION WITH *E. RHUSIOPATHIAE*

By

A. W. Gledhill,

Institute of Animal Pathology, University of Cambridge

INTRODUCTION

The efficacy of antiserum prepared against *E. rhusiopathiae* in the prophylaxis and cure of swine erysipelas is well known. The work to be described was carried out in an attempt to ascertain (*a*) whether strains of this organism are homogeneous in the sense that an antiserum prepared against one strain will protect against all strains; (*b*) whether there is any relation between the potency of immune sera and their agglutinating titres; and (*c*) what kind of antigen is necessary in the preparation of donors for the production of an immune serum.

To elucidate these questions the passive protection was studied that was afforded to mice against infection with virulent strains by immune sera prepared in rabbits and horses.

Though the experience gained in the use of sera in the field rather indicates that the group is homogeneous, Watts (1940) claimed that strains fall into two sub-groups. Each of these contained a heat-stable specific antigen capable of withstanding boiling for four hours and, in addition, all strains possessed at least two common heat labile antigens in varying proportions. The antibodies principally responsible for conferring passive protection corresponded to the heat labile antigens since sera prepared against boiled antigens gave little protection. On the other hand, Julianelle (1941) considers that the group is homogeneous as far as agglutinogens are concerned. Gledhill (1945) showed, however, that strains differ in these respects quantitatively only and to explain the phenomena associated with agglutination there is no need to postulate the existence of heat labile antigens. He also showed that strains may be classified according to their predominant antigen after the manner of Wilson and Miles (1932) for Brucella and that of 31 strains tested, 19 fell into one or other of three groups. It is proposed to refer to these as Group I, II and III; Group IV designates strains which do not fall within these groups.

METHODS

The first essential for protection tests with mice against virulent strains of erysipelothrix is that the animals shall be free from intercurrent disease. In most of the experiments the mice were uniform in size and 20 to 25 g. in weight. In a few of the later experiments the mice were somewhat smaller but this deviation in size appeared to cause no significant difference in the results.

The cultures used for inoculating the protected mice must be highly virulent, capable of killing them in doses of 10^{-8} or 10^{-7} c.c. of a suspension of opacity equal to Brown's tube No. 4. The number of organisms in 1 c.c. of suspension of this latter opacity

is taken as 10^9, which is the approximate figure for *B. coli*. It is well known, too, that strains fluctuate widely in virulence from time to time. Thus Edwards (1931) states that the German official tests for the strength of swine erysipelas serum lay down that the control mice should die within three days. In the experiments to be described the control mice died in three or four days with a dose of culture equal to about 1/10 that used in the German tests. In the later experiments described, however, mice were inoculated with an immune serum shown in the earlier experiments to be unable to protect them unless the virulence of the organism had become somewhat reduced which provided an additional safeguard that the test organism had not decreased in virulence. These precautions are quite essential and on several occasions experiments had to be repeated owing to the attenuated condition of the culture though no difference in technique could be traced to explain its loss in virulence.

Virulent strains were inoculated into two or three mice five days before an experiment was planned to commence. Cultures were made from these mice on the third day and inoculated into glucose broth of *p*H 7·8 which was incubated for 18 hours and centrifuged. The deposit was resuspended in broth and adjusted to opacity equal to Brown's tube No. 4. The dilutions required were made in broth and injected as soon as possible into the serumised mice. Culture suspended in a volume of 0·5 c.c. was injected intraperitoneally into mice which had been inoculated intraperitoneally with serum 24 hours previously. The sera were preserved with merthiolate 1 : 2000, except when it was required to absorb them with living culture, in which case merthiolate was added after absorption to destroy the absorbing culture not removed by centrifuging. Experiments were terminated 14 days after the mice received culture. The observations included, firstly, whether the mice died or survived; secondly, the average survival of batches before death; and, finally, the incidence of sickness in mice that recovered. The details tabulated giving the deaths and average time to die indicate the degree of protection afforded by the immune sera.

In earlier experiments strain EW2 (Group II) was selected for infecting mice because preliminary trials had shown its high virulence. Subsequently, several other strains were employed to confirm and widen the scope of the work. When a strain was required for this purpose it was passaged several times in mice and, provided it was capable of killing them in three days, its M.L.D. was determined to ensure its approximation to EW2. Of 21 strains available, the majority remained incapable of killing mice in three days even after five or six passages, so that they appeared to be permanently unsuitable for protection tests.

From Table I the M.L.D. of EW2 may be taken to be of the order of 100 organisms. It is not improbable that in such high dilutions the errors which arise are considerable, especially when it

TABLE I
THE DETERMINATION OF THE M.L.D. OF STRAIN EW2

Approximate Number of Organisms Injected	*Mice which Died*	*Average time, in days, of death after Inoculation*
50,000	6/6	3·8
5,000	5/5	4·0
500	5/6	4·6
50	5/8	5·8
5	0/4	—

is recalled that great irregularities occur occasionally in the virulence of a given strain. It is doubtful, therefore, if a meaning can be attached to exact multiples of the M.L.D. In the experiments to be described, the doses of culture varied from approximately 100 to 10,000 M.L.D., for the majority the value being 2,000 M.L.D. These doses, it may be remarked, are greatly in excess of the lethal dose of a strain which is ordinarily classed as avirulent, the M.L.D. of which usually contains something in the order of a million times the concentration of organisms in that of EW2.

Preparation of Antisera

Antisera of three kinds have usually been employed: (1) sera prepared with killed antigens, namely, those from rabbits inoculated with antigen grown in glucose broth *p*H 7·8, centrifuged and killed by resuspension in 1 : 2000 merthiolate saline; (2) sera prepared with killed antigens boiled for four hours, referred to as boiled antigen; and (3) sera prepared by further immunising with viable antigen rabbits already immunised with killed antigen. The procedure resembled that of Watts (1940). Doses of 1, 2, 3 and 3 c.c. successively of killed or boiled antigen were injected intravenously into the rabbits, the opacity being standardised to twice that of Brown's tube No. 10. The injections were made at four-day intervals and the rabbits were bled ten days after the last injection. The rabbits further immunised with viable antigens received four injections of 18-hour glucose broth culture in doses of 0·01, 0·1, 1·0 and 1·0 c.c. at weekly intervals and were bled ten days after the last injection. Variations in this technique will be described where necessary.

A COMPARISON OF THE PROTECTION GIVEN BY SERA PREPARED WITH BOILED ANTIGEN, KILLED ANTIGEN AND VIABLE ANTIGEN

If, as Watts (1940) infers, heat labile antigens exist capable of stimulating the production of protective antibodies, then it is of some importance to determine whether a serum containing these antibodies also contains an agglutinin in addition to that evoked by heat-stable antigens alone. If it does not, the generally accepted view that the protective power of a serum is not necessarily proportional to its agglutinating titre is substantiated and explained. To this end, in the first place, a group of mice were tested for

immunity against strain EW2 viable after passive immunisation with serum prepared against EW2 killed, while another group was tested in the same way after immunisation with the same serum that had been absorbed with R (Group I), and hence had all its agglutinins removed. The results obtained as well as those obtained with other groups of mice are shown in Table II. It will be seen that while in the doses used the sera were unable to protect against death, some lengthened definitely the life of the infected mice. This was especially evident with the serum prepared against EW2 killed and the mixture of sera prepared against R killed and EW10 (Group II) boiled.

TABLE II

THE PROTECTIVE POWER OF ABSORBED AND UNABSORBED SERUM PREPARED AGAINST HOMOLOGOUS KILLED ANTIGEN AND OF OTHER SERA

No. of organisms of strain EW2 (Group II) injected	*Serum prepared against:*	*Dose of serum intraperitoneally*	*No. of mice which died*	*Average time to die, in days*
100,000	EW2 (Group II) killed	0·25 c.c.	5/6	7·2
„	EW2, killed absorbed with R, killed	0·25 c.c.	6/6	3·3
„	R (Group I), killed	0·25 c.c.	6/6	4·7
„	EW10 (Group II), boiled	0·25 c.c.	6/6	4·8
„	Mixture of serum R, killed and EW10 boiled	0·125 c.c. of each	6/6	7·0

Four normal controls died in an average time of 3·3 days.

Those tested with the serum against EW2 killed absorbed with R killed died as rapidly as the control mice. The effective constituents of this serum were therefore absorbed by killed culture. Provisionally, this absorbable antibody may be designated "agglutinin." There was some suggestion that the mixture of two sera in half doses gave more protection than full doses of a single serum. To test this point further, mice were protected with sera prepared against each of the four groups and with a mixture of these. The results, shown in Table III, suggest the slight superiority of the mixture and do show that sera prepared with boiled antigen are not markedly inferior to those prepared with antigen killed with merthiolate.

To see whether the effect varied according to the dose of serum employed, a mixture of sera prepared with boiled antigens was injected into separate groups of mice in graded doses. At the same time, groups of mice were injected with a serum prepared by repeated injection of rabbits with killed followed by viable antigen.

TABLE III

A COMPARISON OF THE PROTECTION AFFORDED BY SERA PREPARED WITH BOILED ANTIGENS OF THE FOUR GROUPS

No. of organisms of strain EW2 (Group II) injected	*Serum prepared against:*	*Dose of Serum*	*No. of mice which died*	*Average time to die, in days*
100,000	D boiled (Group I)	0·25 c.c.	5/5	4·6
„	EW10 boiled (Group II)	0·25 c.c.	6/6	5·8
„	EW11 boiled (Group III)	0·25 c.c.	5/6	5·0
„	MEW22, boiled (Group IV)	0·25 c.c.	5/5	5·2
„	Mixture of above 4 sera	0·06 c.c. of each	3/4	8·0
„	—	0·25 c.c. normal serum	8/8	4·6

An 0·25 c.c. dose of the mixed serum—" serum K " (" killed ")—was arbitrarily selected as standard, the estimated equivalent dose for the other being the quantity having an agglutinin content equal to that of the standard of this serum K. The agglutinin content was calculated from the average titre of the sera with strains of the three groups and a Group IV strain (MEW22). " Serum K " consisted of sera prepared with the boiled antigen of strains EW6 (Group I), EW2 (Group II), EW11 (Group III) and MEW22 (Group IV), mixed in equal parts. " Serum V " (" viable ") consisted of a serum prepared by repeated injections in the ordinary way of EW2 killed with merthiolate followed by six weekly injections of a live broth culture of EW2, the doses of the latter being

TABLE IV

THE PROTECTION AFFORDED BY VARYING DOSES OF SERA PREPARED WITH KILLED (K) AND VIABLE (V) ANTIGENS AGAINST INFECTION WITH A CONSTANT NUMBER OF ORGANISMS

Serum used	*Dose employed*	*Mice which died*	*Average time to die, in days*
K	d/3	5/6	5·8
K	d	8/10	6·6
K	3d	4/4	4·8
V	d/3	0/6	—
V	d	1/6	6
V	3d	0/6	—

d refers to the standard dose for the serum in question being, as mentioned above, 0·25 c.c. for K and 0·02 c.c. for V. Six normal controls died in an average time of 3·8 days.

graded from 0·01 c.c. to a final dose of 4·0 c.c. The estimated equivalent dose for this serum was 0·02 c.c. Six mice, as controls, received 0·25 c.c. of normal serum before infection. As before, the infecting dose of culture was 100,000 organisms.

The outstanding fact which the experiment (Table IV) brought to light was the high degree of protection conferred by " serum V " as compared with the serum used in this or any previous experiment.

To confirm this result two rabbits (Nos. 312 and B30) were given four injections of merthiolate-killed EW2, and were bled for serum. They were then injected with graded doses of viable EW2 and again bled for serum. The protective power of the sera from each rabbit was compared, using doses of 0·1 c.c. of serum for each test, the test dose of culture being the same as in the previous experiment. The agglutinating titres of the sera obtained after the injection of live culture were not higher than those of the sera obtained after the preliminary injections of dead culture. The results (Table V) demonstrate the powerful protection conferred by both sera prepared against live culture. With the sera prepared against dead culture only the survival of the mice was considerably lengthened as compared with the controls.

TABLE V

COMPARISON OF PROTECTION CONFERRED ON MICE BY SERA PREPARED FROM RABBITS IMMUNISED WITH KILLED (K) ANTIGENS AND WITH KILLED AND VIABLE (V) ANTIGENS

Serum Rabbit No.	*Immunised with*	*Serum dose*	*Mice which died*	*Average time to die, in days*
312	Dead culture (K)	0·1 c.c.	6/6	7
B30	„	„	6/6	7
312	Live (after dead) culture (V)	„	0/6	—
B30	„	„	2/6	12
Controls	—	—	8/8	2·4

In spite of the high protective power of the sera for mice, both rabbits died within 48 hours of the final bleeding although they had appeared in normal health and were not bled excessively. In one of these rabbits an *Erysipelothrix* septicaemia was demonstrated while cardiac lesions were absent; in the case of the other rabbit, unfortunately, no *post-mortem* examination was conducted.

That the superiority of the serum prepared with viable antigen as compared with the sera prepared with dead antigens was not due to the fact that, in the method employed, the rabbits immunised against viable antigen actually received eight immunising doses, was proved by a further experiment. Two rabbits (722 and 0) were immunised with four doses of killed EW2 and bled in the usual way for serum. One of them (722) was then immunised with four further doses of killed EW2 at four-day intervals, each dose being equal in amount to the last dose of the first immunising course.

The other rabbit (0) was immunised with viable EW2 in the doses as before, also at four-day intervals. Serum was obtained from these rabbits ten days after the last injection. The results (Table VI) confirm those in the preceding experiment.

TABLE VI

FURTHER COMPARISON OF PROTECTION CONFERRED ON MICE BY SERA PREPARED IN RABBITS AGAINST KILLED (K) AND AGAINST KILLED AND VIABLE (V) ANTIGENS

Serum Rabbit No.	*Immunised with*	*Serum dose*	*Mice which died*	*Average time to die, in days*
		c.c.		
722 and 0 (pooled)	Killed EW2 (4 doses) (K)	0·1	5/6	8·6
722	Killed EW2 (8 doses) (K)	0·05	9/12	8·3
0	Killed EW2 (4 doses) followed by viable EW2 (4 doses) (V)	0·05	0/12	—

The infecting dose of EW2 was 100,000 organisms and the five normal controls died in 3·8 days. The doses of immune serum are in inverse proportion to their agglutinin content estimated with killed EW2 antigen.

To determine the protective power of sera against varied doses of infecting organisms, the two sera obtained from rabbit B30 (Table V) were inoculated into mice at a constant dose of 0·25 c.c., the infecting strain, as before, being EW2. The results show (Table VII) that over a wide range of infecting doses there is no relation between the degree of protection and the infecting dose. This is in agreement with the findings of Viriden (1933), who states that such a relationship is only apparent when the prophylactic dose of serum is near the minimal and the infecting dose is very large.

TABLE VII

THE PROTECTIVE POWER OF IMMUNE SERA AGAINST VARYING INFECTING DOSES

No. of organisms of strain EW2 (Group II) injected	*Serum from B30 prepared with killed EW2*		*Serum from B30 prepared with viable EW2*	
	Mice which died	*Average time to die, in days*	*Mice which died*	*Average time to die, in days*
10,000 (10^2 M.L.D.)	4/6	8·0	Not tested	—
100,000 (10^3 M L D)	2/4	6·5	Not tested	—
1,000.000 (10^4 M L.D)	5/6	8·0	2/6	5·0
10 000,000 (10^5 M.L.D.)	5/6	6·4	0/6	—

The dose of serum throughout was 0·25 c.c. The controls died in an average time of 3·0 days.

IMMUNE PROPERTIES OF RABBIT SERA PREPARED WITH VIABLE CULTURE

The protection afforded by serum prepared with viable EW2 after the absorption of agglutinins.—Serum was prepared with killed EW2 (*i.e.*, Group II) followed by living EW2, as already described. It was then absorbed with EW2 boiled and EW6 (Group II) boiled, after which it no longer agglutinated merthiolate-killed suspensions of strains EW2, EW6, EW11 (Group III) or MEW22 (Group IV). The protective power for mice of this absorbed serum alone and mixed with serum prepared against another strain (D) (Group I) boiled, as a source of agglutinins, is shown in Table VIII. Although strain D belonged to Group I, the serum prepared against it as boiled antigen possessed a high agglutinating titre with strains of Group II. The other sera were included in the test for comparison and as a check on the virulence of the infecting culture. It is evident that the absorbed serum contained some persisting protective antibody (X).

TABLE VIII

THE PROTECTION AFFORDED BY SERUM PREPARED WITH VIABLE EW2 AND ABSORBED WITH BOILED ANTIGENS

Serum prepared against:	*Details of Sera*	*Dose of Serum*	*Mice which died*	*Average time to die, in days*
		c.c.		
EW2 (viable) (Group II)	Absorbed with EW2 and EW6 boiled	0·05	4/6	10·8
EW2 (viable) (Group II)	Absorbed with EW2 and EW6 boiled	0·05	2/6	9
+ D (boiled) (Group I)		0·25		
D (boiled) (Group I)		0·25	6/6	4
EW2 (viable) (Group II)		0·05	1/12	9

The infecting dose was 100,000 organisms of strain EW2. The four controls died in an average time of 3·5 days after infection.

The above serum prepared against viable EW2 was next absorbed with merthiolate-killed, instead of boiled, cultures. Again, a serum was prepared with killed EW2 followed by viable EW2, and to it no preservative was added. It was then absorbed with living culture of the same strain. The results of tests upon mice (Table IX) prove that the protective antibody X was not completely absorbed in either case. Whether it was really absorbed at all is a question which is not easy to solve as some loss of antibody X may have taken place during absorption. Mice which receive serum absorbed in any way usually develop a transient illness with diarrhoea as a prominent symptom, and this toxic change in the property of the serum may

possibly account for the observed inferiority of such serum as compared with equivalent unabsorbed serum. It should be stated that early workers noticed that there is no absorption of immune bodies as a result of the direct contact of living organisms with immune serum (Edwards, 1931).

These experiments (Tables VIII and IX) also provide some evidence which is further supported later that a mixture of serum prepared against boiled or merthiolate-killed antigen and of absorbed serum prepared with living antigen gives better protection than such an absorbed serum alone. It has been shown, however, that sera

TABLE IX

THE ABSORPTION OF ANTIBODY X BY MERTHIOLATE-KILLED EW2 AND BY VIABLE EW2

Serum prepared against:	*Details of Sera*	*Dose of Serum*	*Mice which died*	*Average time to die, in days*
		c.c.		
EW2 (viable) (Group II)	Absorbed with killed EW2	0·05		
			0/6	—
D (boiled) (Group I)		0·25		
D (boiled) (Group I)		0·25	4/6	6
EW2 (viable)	Absorbed with viable EW2	0·025	5/5	6
EW2 (viable)	As above	0·025		
			1/6	6
EW2 (killed-merthiolate)		0·10		
EW2 (killed-merthiolate)		0·10	6/6	4·7

The infecting dose was 100,000 organisms of EW2 strain. The three controls died in an average time of 2·7 days.

prepared with boiled antigens do not protect mice, although those prepared with merthiolate-killed antigens gave slight protection. The result can perhaps be explained as due to immune body in subliminal amounts in sera prepared against boiled, and killed, antigens which, when added to an absorbed serum containing immune body in liminal amounts, produces a pooled serum giving definitely better protection than an absorbed serum alone. Against this explanation the following considerations may be urged: (1) that a serum prepared against killed antigen lost its power to delay the death of infected mice after absorption (Table II), whereas the immune body produced with live culture was not absorbed completely; and (2) that since immune body is produced to a much greater extent by live than by killed antigen, it may be inferred that the effective antigen is labile.

In that case, therefore, it is difficult to understand why immune body capable of lengthening the survival of infected mice is produced equally against antigen which has been boiled for four hours as against antigen killed with merthiolate. These difficulties are evaded if it is supposed that boiled, killed and viable antigens produce an easily absorbable antibody which itself can only delay the death of infected mice but which can support the action of the antibody produced abundantly against live antigen. For convenience, the immune body produced with living culture will be termed antibody X without prejudice to the question of whether or no it is produced in small amounts with killed antigen.

The cross protection afforded by immune sera.—The primary object of the experiment (Tables X, XI and XII) was to ascertain whether the protective antibody produced with viable EW2 protects against other virulent strains of *Erysipelothrix* and, conversely, whether the serum produced by another such strain protects against EW2. As already said, only a few of the strains available were sufficiently virulent for mice to give satisfactory results on test. Two strains, Ru (Group I) and V (Group III), corresponding to the groups in which EW6 and EW11 fell, respectively, were isolated from pigs infected with erysipelas, and proved to be highly virulent for mice. A third strain, EW24 (Group II), which by good fortune was found to be sufficiently virulent, was selected because it originated

TABLE X

THE PROTECTION CONFERRED BY SERUM FROM RABBITS AGAINST EW2 (GROUP II) UPON MICE INFECTED WITH RU STRAIN (GROUP I)

No. of organisms of strain Ru (Group I) injected	*Serum prepared against*	*Details of Serum*	*Dose of Serum*	*Mice which died*	*Average time to die, in days*
100,000	EW2 (viable) (Group II)		c.c. 0·05	0/6	—
100,000	EW2 (Group II)	Serum as above absorbed with boiled EW2 antigen	0·05	3/6	12·3
100,000	EW2 (Group II)	Absorbed serum as above	0·05	0/6	—
	D (boiled) (Group I)		0·25		
100,000	D (boiled) (Group I)		0·25	6/6	6·2

All four controls died in an average time of 4·8 days.

from Japan, and it has been claimed by Lehnert *et al.* (1942) that sera prepared against Danish, French and American strains do not protect mice as satisfactorily against infection with Japanese strains as against infection with European and American strains. The M.L.D.s of V and Ru strains were such that doses of 500 organisms killed mice, which represents a degree of virulence equal to that of EW2. In the case of EW24 the virulence was not determined accurately owing to the shortage of suitable mice at the time, but it appeared to be as virulent as the other three judging from the rapidity with which it killed mice in moderate doses. The results show that the protective antibody X produced by viable EW2 protects strongly though not completely against all three strains. Some stronger support is given to the previous indication of an ancillary function of agglutinins in protection.

TABLE XI

THE PROTECTION AFFORDED BY SERUM PREPARED WITH EW2 (GROUP II) TO MICE INFECTED WITH V STRAIN (GROUP III)

No. of organisms of strain V (Group III) injected	*Serum prepared against*	*Details of Serum*	*Dose of Serum*	*Mice which died*	*Average time to die, in days*
			c.c.		
	EW2 (viable) (Group II)	Absorbed with killed EW2	0·01	1/6	9·0
100,000					
	MEW22 (boiled) (Group IV)		0·10		
100,000	MEW22 (boiled) (Group IV)		0·10	6/6	5·0

All controls died in an average time of 3·3 days.

TABLE XII

THE PROTECTION AFFORDED BY SERUM PREPARED WITH EW2 (GROUP II) TO MICE INFECTED WITH EW24 STRAIN (GROUP II, JAPANESE)

No. of organisms of strain EW24 (Group II) injected	*Serum prepared against*	*Dose of Serum*	*Mice which died*	*Average time to die, in days*
		c.c.		
1,000,000	EW2 (viable)	0·1	1/6	12·0
1,000,000	EW2 (killed)	0·1	6/6	4·3

All six controls died in an average time of 3·0 days.

The protection afforded by sera prepared with strains not related to the infecting strain is further shown in Table XIII. Serum K was prepared in a rabbit with killed EW6 (Group I). Serum V was prepared by further injecting into this rabbit graded living doses of Ru strain (Group I). Strain Ru was of higher virulence than EW6 and was chosen to follow killed EW6 because at this stage it was not known whether or not the production of protective antibody depended on the virulence of the strain used.

TABLE XIII

THE PROTECTION AFFORDED BY SERA PREPARED WITH KILLED (K) EW6 AND VIABLE (V) RU (BOTH GROUP I) AGAINST INFECTION WITH EW2 (GROUP II)

No of organisms of strain EW2 (Group II) injected	*Serum employed*	*Dose of Serum*	*Mice which died*	*Average time to die, in days*
100,000	K (EW6, Group I)	c.c. 0·1	6/6	4·5
100,000	V (Ru, Group I)	0·01	3/6	11·0
100,000	Normal	0·1	4/6	3·0

The serum prepared with viable culture gave better protection, as might have been anticipated. The noteworthy point is, however, that viable Ru strain evidently produces an antibody that protects, partially at any rate, against EW2, while viable EW2 (see Table X) produces an antibody which likewise protects against Ru.

Are avirulent strains capable of producing protective antibody X *in the same way as virulent strains?*

Three rabbits were immunised with killed EW2, the usual virulent strain. Sera were obtained from these and then they were further immunised with viable antigens, one with EW2, one with "Antwerp" (Group I) and one with 1224 (Group I). The "Antwerp" strain had an M.L.D. about a million times larger than that of EW2; the M.L.D. of 1224 was not determined but there was little reason to believe it differed greatly from that of "Antwerp" since 12 mice all remained normal after receiving amounts equal to about 1,000 M.L.D. of EW2. Six groups of mice were infected with EW2 in the usual dose after being passively immunised respectively with the three sera against killed EW2 and the three sera against the viable organisms (Table XIV). The doses of each kind of serum given were in inverse proportions to their agglutinating titres against EW2. The avirulent strains proved as effective as the virulent strains in the production of protective antibody X. As indicated by the serum

dosage the avirulent strains did not provoke as good an agglutinin response as EW2; this is probably associated with the fact that they were partly rough, particularly "Antwerp." In Table XIV is also included a test of a serum prepared by injecting a normal rabbit with living bacilli of strain 1224 in four doses of 0·01 c.c., 0·1 c.c., 1·0 c.c. and 1·0 c.c. at four-day intervals and bleeding ten days after the last injection. This result is of importance as showing the power of viable avirulent strains to stimulate an effective antibody response. It also further substantiates the conclusion that the superiority of sera prepared with killed culture followed by viable culture over those prepared with killed culture only is actually due to subsequent immunisation with living organisms.

TABLE XIV

THE IMMUNISING POWER OF VIABLE AVIRULENT STRAINS

Serum prepared against:	*Serum Rabbit No.*	*Dose of Serum*	*Mice which died*	*Average time to die, in days*
		c.c.		
Killed EW2	544	0·025	16/18	3·8
"	546	0·025		
"	547	0·0125		
Killed EW2 + viable EW2	544	0·0125	0/6	—
Killed EW2 + viable Antwerp	546	0·05	0/6	—
Killed EW2 + viable 1224	547	0·05	0/6	—
Viable 1224 alone	737	0·10	1/6	8·0

All five controls died in an average time of 2·6 days.

EXPERIMENTS WITH SERA PREPARED IN THE HORSE

Three sera were kindly supplied by Dr. Montgomerie, of the Wellcome Research Laboratories. They were prepared by the injection of horses with killed organisms followed by graded doses of broth culture containing living organisms. Two of the sera were prepared with strain HSE11 (Group I) and the other with strain HE (Group II). Mice were immunised with these sera alone, with these sera absorbed with antigens of the four groups and with these absorbed sera to which was added a serum (P) prepared in a rabbit against a killed Group IV strain (MEW22) which possessed a high agglutinating titre against strains of all the groups. The purpose of adding this latter serum was to restore to the absorbed horse sera agglutinins which appear to act in an ancillary way to enhance the protective power of sera (Tables VIII and IX). It would have been more satisfactory to have added a serum prepared in the horse against killed antigen but unfortunately one was not available at the time.

The infecting dose was 100,000 organisms of EW2 strain. The results of protection experiments with these sera (which are referred to as HSEIIa, HSEIIb and HE) are shown in Table XV.

TABLE XV
THE PROTECTION AFFORDED BY SERA FROM HORSES IMMUNISED WITH VIABLE ORGANISMS

Serum	*Details of Sera*	*Dose of Serum*	*Mice which died*	*Average time to die, in days*
		c.c.		
HSEIIa (Group I)	Prepared with viable HSEII in horse	0·1	1/6	12·0
HSEIIa (Group I)	Absorbed with killed antigens	0·1	5/5	3·0
HSEIIb (Group I)	Prepared with viable HSEII in horse	0·04	2/6	8·5
HSEIIb (Group I)	Absorbed with killed antigens	0·04	4/4	5·5
P (Group IV)	Prepared with boiled MEW22 in rabbit	0·10		
HE (Group II)	Prepared with viable HE in horse	0·04	1/6	11·0
HE (Group II)	Absorbed with killed antigens	0·04	6/6	5·0
HE (Group II)	Absorbed with killed antigens	0·04	6/6	6·5
P (Group IV)	Prepared with boiled MEW22 in rabbit	0·10		
P	As above	0·10	4/6	5·3

Twelve controls died in an average time of 2·7 days.
N.B.—The agglutinating titres of these three horse sera were of the same order as the rabbit sera considered above.

The results are not entirely in accord with those obtained in the case of rabbit sera prepared with viable organisms for, though the horse sera give good protection when unabsorbed, absorption would appear to remove the antibodies completely. The most obvious explanation of this difference is that antibody X, which has been shown to be difficult to absorb from rabbit sera, is comparatively easily absorbed from horse sera. This would probably mean that horse antibody X is not exactly the same as rabbit antibody X, but this would not be surprising. That the protective mechanism of horse immune serum is not of a fundamentally different kind is suggested by the fact that viable culture is used in the production of sera for use in the field.

An experiment was carried out to compare the protection afforded to mice by a horse serum prepared with merthiolate-killed EW2 with the serum obtained by further immunising the same horse with viable EW2. The technique resembled that already employed for rabbits, except that the immunising doses were much larger. Five injections of killed antigen were given at four-day intervals, the doses ranging from 1 c.c. to 10 c.c. of a suspension standardised to Brown's tube No. 10, followed by four doses of viable antigen ranging from 0·5 c.c. to 10 c.c. of 18 hour glucose broth culture. The horse was bled for serum eight days after each set of injections. The resulting sera did not protect mice against the usual dose of EW2 but against a dose of 1/10 of this size, that is about 200 M.L.D., it was found that only the serum from the horse after immunisation with live culture gave protection as in the case of sera from rabbits. (Table XVI.)

TABLE XVI

No. of organisms of strain EW2 injected	*Serum prepared in horse against*	*Dose of Serum*	*Mice which died*	*Average time to die, in days*
		c.c.		
10,000	EW2 dead	0·3	6/6	4·8
„	EW2 dead + viable EW2	0·15	0/6	—

All six controls died in an average time of 4·0 days. The serum dosage is inversely proportional to the agglutinating titres.

DISCUSSION

The experiments described demonstrate clearly that in the rabbit at any rate an antibody is produced by viable antigen which is not produced by killed antigen in significant amount and that this antibody is largely responsible for the protection afforded by sera prepared in this way. The antibody was partly but not completely absorbed from immune rabbit sera. Some indication was obtained that it is more effective in the presence of the antibodies found in sera prepared with killed antigen and reasons have been advanced for supposing that these antibodies are not just antibody X present in small amounts. Assuming the existence of easily absorbable antibodies, these certainly include agglutinins and they have been referred to as agglutinins for convenience. The evidence brought forward does not establish that they are agglutinins exclusively though it is not inconsistent with this view. Since the work of Dean (1917) there has been a tendency to regard agglutinins, opsonins, precipitins and lysins as different actions of a single antibody. Gajzago (1932) has shown that specific opsonins play an active role in the protective power of immune sera so that it can be inferred from the evidence available that the easily absorbable antibodies responsible for protection in sera prepared with killed antigens are probably identical with agglutinins provided it can be assumed that opsonic

action and agglutination are manifestations of a single antibody. That these antibodies are agglutinins is further indicated by the fact that they are produced by antigens which, like agglutinogens, resist boiling for four hours (compare Tables II and III). Doubtless the reason why, in the experiments cited, immune sera prepared with killed antigen did not give complete protection to mice is attributable to the large multiple of the M.L.D. of live antigen used to infect them. In preliminary trials, smaller doses were employed and in these cases sera against killed antigens frequently gave complete protection though this procedure was abandoned because the results were erratic.

SUMMARY

Mice that had been passively immunised with sera prepared in various ways in rabbits and horses were tested for protection with various, serologically different, strains of *Erysipelothrix* with the following results :—

(1) No evidence was obtained to warrant the belief that an immune serum prepared against one strain would fail to set up protection against other, that is, serologically classifiable different strains.

(2) To obtain an effective immune serum it was essential to use a viable antigen to inoculate the donors ; an effective serum was obtainable whether the viable antigen was of high or low virulence.

(3) When the immune serum was prepared from rabbits, its protective efficacy was shown to be due to the presence of antibody which could not be easily absorbed completely.

(4) Antibodies easily removable from immune rabbit sera by absorption were probably O-agglutinins.

(5) These easily removable antibodies failed to protect mice against fatal infection with the doses of infective organisms used for test though they somewhat lengthened survival. They also enhanced appreciably the action of the antibody which could not be completely absorbed that was produced by viable antigens.

(6) Horse immune sera behaved like the rabbit immune sera except that their immune antibodies could be completely removed by absorption.

REFERENCES

Dean, H. R. (1917). *Lancet*, **1**, 45.
Edwards, J. T. (1931). *M.R.C. System of Bacteriology*. Vol. 8, p. 379. London : H.M. Stationery Office.
Gajzago, D. (1932). *Zbl. Bakt.* I (Orig.), **125**, 89.
Gledhill, A. W. (1945). *J. Path. Bact.* (in press).
Julianelle, L. A. (1941). *J. Bact.*, **42**, 385.
Lehnert, E., Toll, H., and Bodins, S. (1942). *Z. InfektKr. Haustiere*, **58**, 226. Abstract : *Vet. Bull.*, **13**, 82.
Viriden, P. (1933). *Skand. vet. Tidskr.*, **23**, 421.
Watts, P. S. (1940). *J. Path. Bact.*, **50**, 355.
Wilson, G. S., and Miles, A. A. (1932). *Brit. J. exp. Path.*, **13**, 1.

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HISTOLOGICAL EVIDENCE INDICATING THAT A FORM OF EQUINE ENCEPHALOMYELITIS OCCURS IN PALESTINE

By

I. H. Pattison

Government Veterinary Laboratory, Tel-Aviv, Palestine.

INTRODUCTION

As far as the writer is aware, the only published record of a nervous disease of equines in Palestine that might be diagnosed clinically as encephalomyelitis is that of Firun (1939), who described two cases in police horses of what he called "a disease with cerebral symptoms." In one case there was recovery and in the other the animal died. Examination was confined to clinical observation and autopsy on the dead animal, and in Firun's opinion the disturbances "may have resulted from toxic substances present in spoiled food." Firun drew attention to the fact that the cases observed by him were clinically similar to those described by Cox, Philip, Marsh and Kilpatrick (1938) in an outbreak of a disease among horses in Western Montana, in which efforts to isolate an aetiological agent or to demonstrate histological lesions in the central nervous system failed.

HISTORY OF THE DISEASE IN PALESTINE

Since the observations of Firun, the first case suggesting that a form of equine encephalomyelitis might exist in Palestine came to the notice of the Government Veterinary Laboratory in October, 1940, when a horse belonging to the Army showed symptoms that were diagnosed clinically as encephalomyelitis. The animal was destroyed and brain material was inoculated into several guinea-pigs with negative results. The following month a second and very similar case occurred, again in an Army horse, but again attempts to infect guinea-pigs were unsuccessful. At this time Army veterinary surgeons had under their care several animals showing nervous symptoms in varying degree, but none sufficiently far advanced to warrant destruction. The last available clinical report concerning these animals stated that they were "all on the way to recovery."

In June, 1941, another case of suspected encephalomyelitis in an Army horse was reported to the laboratory, but no material suitable for establishing a positive diagnosis was submitted. During the latter part of that summer what amounted to a mild epizootic occurred, again, as far as is known, confined to Army equines. At

least 30 animals were involved of which one died, 18 were destroyed, one was cured and ten remained neither cured nor sufficiently useless to be destroyed. The later histories of these ten animals are not known. The clinical symptoms shown by animals involved in this epizootic varied widely, but the following is a brief description of the typical course of the disease. Sudden onset; at first nervousness and hyperexcitability increased by any external stimulus such as a loud noise or sudden movement; almost continuous twitching of the lips, ears and eyelids; inco-ordination of movement and weaving of the body; staggering when forced to turn and marked knuckling of the fetlocks when stopped suddenly; in some cases complete paralysis of the hindquarters. In no case was a high temperature recorded and generally it was within the normal range; usually the appetite was unimpaired.

Material from the central nervous system of two animals involved in this outbreak was available for histological examination, and the findings are reported in this paper. (Case 1 and Case 2.)

In the spring of 1942 occasional cases of a " paralytic disease " in Army units occurred among French horses recently brought from Syria. Clinically, these cases resembled those that had occurred in the above-mentioned epizootic some six months previously. Only one English horse was involved. No material from these cases was received at the laboratory.

In the late summer of 1942 a second mild epizootic of what appeared to be the same disease as that described above occurred among horses belonging to the Army and accommodated in the same veterinary hospital as that in which the first epizootic had occurred. Again at least 30 animals were involved. Symptoms were: sudden onset; unsteadiness of gait; partial or complete paralysis of the limbs especially the hind-limbs; little, if any, rise in temperature. The mortality was low and some animals appeared to recover completely.

Part of the brain of one animal destroyed during this epizootic was available for histological examination (Case 3).

During this outbreak the possibility that the disease was due to " forage-poisoning " or to some form of entero-toxaemia was investigated, with negative results.

The remaining cases reported on in this paper occurred in mules belonging to the Army, two of which had been imported from the Sudan and one from Eritrea shortly before clinical symptoms appeared. Two were destroyed in May, 1943, and one in June, 1943, as being too lame to be of any further use. One of these animals (Case 4) had been showing symptoms for five days before destruction, and this case may be considered clinically subacute. The remaining two (Case 5 and Case 6) had been showing symptoms for three months and one month respectively.

HISTOLOGICAL METHODS

Table I indicates the origin of the material examined histologically.

TABLE I
ORIGIN OF MATERIAL EXAMINED HISTOLOGICALLY

Case No.	*Animal.*	*No. days ill*	*Clinical*
1 1941 epizootic	Horse	2	Acute case; hind limbs paralysed; unable to rise; destroyed.
2 1941 epizootic	Horse	?	Acute case; destroyed *in extremis*.
3 1942 epizootic	Horse	3	Acute case; hind limbs paralysed; unable to rise; destroyed.
4 Sporadic case 1943	Sudanese mule	5	Sub-acute case; weakness of hind limbs; first symptoms noticed immediately on arrival by train from the Sudan; destroyed.
5 Sporadic case 1943	Sudanese mule	At least 90	Chronic case; very unsteady behind; atrophy of loin and thigh muscles; first symptoms two months after arrival from the Sudan; destroyed.
6 Sporadic case 1943	Eritrean mule	30	Chronic case; unsteady behind spasmodic jerking while standing; destroyed.

After fixation in 10 per cent. formol-saline, tissues were dehydrated in alcohols of increasing strength, cleared and hardened in chloroform and embedded in paraffin. The routine staining method used was haematoxylin and eosin, but selected sections from three animals were stained by the method described by Smith and Quigley (1937), in an effort to demonstrate myelin degeneration.

No special method for demonstrating inclusion bodies in nerve cells was used, but it may be noted that Negri bodies in a known case of rabies were stained quite clearly by this routine haematoxylin-eosin technique.

HISTOLOGICAL FINDINGS

In order to avoid repetition and to make the following descriptions as brief as possible, it is to be noted that in every case the cells involved in vascular and perivascular infiltration were (*a*) lymphocytes; (*b*) larger mononuclear cells; (*c*) occasional plasma cells. No polymorphonuclear leucocyte was found in any section. (See Fig. 2.)

Following the descriptive method of Seifried (1934), a distinction is made between vascular infiltration and perivascular infiltration. The term vascular infiltration signifies that the infiltrating cells are confined to the perivascular lymph channels; by perivascular infiltration is meant an infiltration of cells into the perivascular lymph channels and also, to a lesser or greater extent, into the surrounding tissues.

Clinically acute cases

Case 1.

Parietal cortex.—Slight vascular infiltration of occasional small vessels in the white matter; occasional nerve cells showing slight degenerative changes; no evidence of reaction of glial cells; no meningeal reaction. *Hippocampal cortex.*—No marked abnormality noted; occasional pyramidal cells showing slight degenerative changes. *Cerebellum.*—One small area of focal infiltration of cells in the grey matter, otherwise no abnormality noted. *Medulla.*—Slight vascular and perivascular infiltration of some small vessels; numerous degenerating nerve cells; occasional small areas of cell infiltration in the brain substance; no meningeal reaction noted. (See Fig. 3.)

Case 2.

Parietal cortex.—Slight vascular infiltration of small vessels in both grey and white matter; moderate number of nerve cells showing degenerative changes; neuronophagia noted; no meningeal reaction. *Hippocampal cortex.* — Vascular and perivascular infiltration of occasional vessels; numerous degenerating pyramidal cells; no meningeal reaction noted. *Cerebellum.*—Slight vascular infiltration of one small vessel in the white matter, otherwise no abnormality noted. *Cervical cord.*—Well marked vascular and perivascular infiltration of numerous vessels in both grey and white matter; many small areas of focal infiltration in grey and white matter; many degenerating nerve cells; no meningeal reaction. Myelin sheath staining failed to show evidence of myelin degeneration.

Case 3.

Pons.—Vascular and perivascular infiltration of many small and larger vessels; many small areas of focal tissue infiltration; numerous degenerating nerve cells; neuronophagia noted; no meningeal reaction. *Anterior medulla.* Vascular and perivascular infiltration quite marked; small areas of focal tissue infiltration; about 50 per cent. of the nerve cells appear to be undergoing degenerative changes. *Posterior medulla.*—As for anterior medulla; no meningeal reaction noted.

Clinically subacute case

Case 4.

Frontal cortex.—Slight vascular infiltration of occasional small vessels in the white matter; occasional nerve cells showing degenerative changes; neuronophagia noted; no evidence of meningeal reaction. *Occipital cortex.*—As for frontal cortex. *Hippocampal cortex.*—Marked vascular and perivascular infiltration of numerous vessels, especially in the white matter; small foci of infiltrating cells in grey and white matter; many degenerating pyramidal cells. *Cerebellum.*—Slight vascular infiltration of occasional

small vessels in the white matter. *Medulla.*—Marked vascular and perivascular infiltrations and many areas of focal infiltration in the brain substance; many degenerating nerve cells; no meningeal reaction noted. Myelin sheath staining showed no evidence of demyelination. (See Figs. 1 and 2.) *Cervical cord.*—As for medulla.

Clinically chronic cases

Case 5.

Frontal cortex.—Occasional pyramidal cells showing slight degenerative changes, otherwise no abnormality noted. *Parietal, Occipital and Hippocampal cortex.*—As for frontal cortex. *Basal ganglia and Mid-brain.*—No abnormality detected. *Pons.*—Occasional nerve cells showing slight degenerative changes. Staining by a myelin sheath stain failed to show evidence of myelin degeneration. *Cerebellum.*—No abnormality detected. *Anterior medulla.*—Occasional degenerating nerve cells, otherwise no abnormality detected. *Posterior medulla.*—Degenerating nerve cells rather more numerous than in the anterior medulla, but degenerative changes not marked. Slight meningeal reaction. *Cervical cord.*—Vascular and perivascular infiltration of numerous small vessels in grey and white matter; small foci of infiltrating cells in grey and white matter of the cord substance; degeneration of nerve cells in both dorsal and ventral horns; slight meningeal reaction. *Thoracic and Lumbar cord.*—As for cervical cord. (See Fig. 4.)

Case 6.

Frontal, Parietal, Occipital, Hippocampal cortex and Basal ganglia.—No abnormality detected. *Mid-brain.*—Rare nerve cells showing slight degenerative changes, otherwise no abnormality detected. *Pons* and *Posterior medulla.*—As for mid-brain. *Cerebellum and Anterior medulla.*—No abnormality detected. *Cervical cord.*—Degeneration of numerous nerve cells in both dorsal and ventral horns; slight vascular infiltration of occasional small vessels; no meningeal reaction. *Thoracic cord.*—Slight vascular infiltration of several small vessels in the white matter; occasional foci of infiltrating cells in the white matter; degenerative changes in nerve cells of dorsal and ventral horns, but this is less marked than in the cervical cord; no meningeal reaction. *Lumbar cord.*—Degenerative changes in occasional nerve cells; vascular infiltration of one small vessel; no meningeal reaction.

Summary of histological findings

(*a*) In the clinically acute and subacute cases a generalised inflammatory reaction was seen throughout the brain, characterised by vascular and perivascular infiltration of small blood vessels with lymphocytes, larger mononuclear cells and occasional plasma cells, small foci of infiltrating cells in grey and white matter and degenerative changes in nerve cells. (*b*) In clinically chronic cases a similar inflammatory reaction and degenerative changes in nerve cells confined almost entirely to the spinal cord. (*c*) Grey and white matter appear to be affected to an equal degree. (*d*) Slight meningeal reaction was noted in one of the chronic cases. (*e*) Staining of selected sections from three cases with a myelin sheath stain failed to reveal evidence of myelin degeneration. (*f*) No inclusion bodies, demonstrable by the staining methods employed, were found in nerve cells.

It is realised that the interpretation of the significance of occasional apparently degenerating nerve cells in the central nervous system of animals is extremely difficult. They may be found in a wide variety of pathological conditions (many apparently unconnected directly with the central nervous system), and also in animals that have been destroyed in apparent good health. Therefore, in the above examination, nerve cell degeneration is only mentioned in cases where little apparent doubt existed that the degree of degeneration or the number of affected cells was of pathological significance.

ATTEMPTED TRANSMISSION OF THE DISEASE TO LABORATORY ANIMALS

Material from Case 4 was available for transmission experiments. The inoculum used was a bacteriologically sterile Chamberland L3 filtrate prepared from a saline suspension of cerebrum, cerebellum and medulla after storage for nine days in 50 per cent. glycerine-saline. The inoculations carried out are enumerated below. None of the animals contracted the disease.

Animal	*Dose of inoculum*	*Method of inoculation*
2 rabbits	0·4 c.c.	Intracerebrally
1 rabbit	5·0 c.c.	Subcutaneously
1 „	2·0 c.c.	Intravenously
4 guinea-pigs	0·3 c.c.	Intracerebrally
1 guinea-pig	3·5 c.c.	Subcutaneously
1 „	2·0 c.c.	Intracardially
7 mice	0·1 c.c.	Intracerebrally
1 mouse	0·4 c.c.	Intravenously
1 „	0·2 c.c.	„
1 „	0·5 c.c.	Subcutaneously

DISCUSSION

Equine encephalomyelitis has been reported by numerous workers from various parts of the world, *e.g.*, Germany, France, Russia, Rumania, Hungary, Yugoslavia, the United States of America, Mexico, Venezuela, India, the Dutch East Indies, and it is now generally recognised (Hutyra, Marek and Manniger, 1938) that at least two forms of the disease exist, namely (*a*) Borna disease (so-called enzootic meningo-encephalomyelitis simplex), and (*b*) so-called enzootic haemorrhagic meningo-encephalomyelitis. It has been suggested, however (Meyer, Haring and Howitt, 1931) that the form of encephalomyelitis met with in the United States of America is

also a separate disease and recent work carried out in the U.S.A. appears to justify this opinion.

It is impossible to draw final and very definite conclusions regarding equine encephalomyelitis in Palestine from a histological and clinical examination that is admittedly incomplete. However, the available data indicate firstly that the disease differs from Borna disease, and secondly, that it differs from the disease described in the U.S.A. In Borna disease (*a*) a high mortality rate is the rule and prognosis is always most unfavourable ; (*b*) intranuclear inclusion bodies in the nerve cells are easily demonstrable in almost every case ; and (*c*) according to Seifried, the grey matter of the brain suffers greater damage than does the white matter. These facts indicate that the disease in Palestine is not identical with Borna disease.

Hurst (1934) has described the histological picture in American equine encephalomyelitis due to the eastern virus as a diffuse, acute inflammation of the central nervous system affecting especially the grey masses with primary degeneration of nerve and neuroglial cells, followed by a heavy tissue infiltration with polymorphonuclear leucocytes ; reactive changes in the microglia ; nuclear inclusions rather similar to those in Borna disease ; finally perivascular infiltration that is either predominantly polymorphonuclear or wholly mononuclear. In a horse artificially infected with the western virus he observed lesions that were individually the same as in the eastern virus cases, but far less numerous and intense. This description indicates that, on a histological basis at least, the disease in Palestine is not identical with the disease in America.

Shirlaw (1940) has reviewed extensively the type of encephalomyelitis that occurs in India and has concluded that the disease in that country is a specific virus infection. He has pointed out a similarity between the disease observed by him and a form that has been described in France. The disease that apparently exists in Palestine shows many points of similarity with that described by Shirlaw, although it has not been investigated as thoroughly as has the disease in India. The main point of difference between the two descriptions is that haemorrhages do not appear to be a feature of the disease in Palestine. The inability, to date, to transmit the Palestine form of the disease to laboratory animals does not necessarily greatly lessen the similarity between this and the Indian form of the disease, because in the latter attempts at transmission have only been successful in a certain proportion of cases.

The fact that a form of equine encephalomyelitis may be considered to exist in Palestine is of considerable interest in the light of the recent observation of Bennett (1942), based, as he says, upon circumstantial evidence, that equine encephalomyelitis exists in the Sudan. Indeed, it is by no means impossible that two of the sporadic cases in mules reported on in this paper (Cases 4 and 5) were actually infected with the disease before they entered Palestine.

SUMMARY

Histological findings in the central nervous system of six equines affected during life with symptoms clinically resembling equine encephalomyelitis, are described.

It is concluded that a form of equine encephalomyelitis occurs in Palestine.

The possibility is discussed that this form of equine encephalomyelitis differs from Borna disease and from the form met with in the United States of America, but resembles the type described in India.

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REFERENCES

Bennett, S. C. J. (1942). *Ann. Rep. Sudan Vet. Service*, 22.
Cox, H. R., Philip, C. B., Marsh, H., and Kilpatrick, J. W. (1938). *J. Amer. vet. med. Ass.*, **93,** 225.
Firun, N. (1939). *Vet. J.*, **95,** 142.
Hurst, E. W. (1934). *J. exp. Med.*, **59,** 529.
Meyer, K. F., Haring, C. M., and Howitt, B. (1931). *J. Amer. vet. med. Ass.*, **79,** 376.
Shirlaw, J. F. (1940). *J. Roy. army vet. corps*, **11,** 40 and 75.
Smith, W. K., and Quigley, B. (1937). *Amer. J. Path.*, **13,** 491.

Works consulted

Hutyra, F., Marek, J., and Manniger, R. (1938). "*Special Pathology and Therapeutics of the Diseases of Domestic Animals.*" 4th ed. Baillière, Tindall and Cox, London:
Seifried, O. (1934). "*Lehrgang der Histopathologie für Studierende und Tierärzte.*" Julius Springer, Berlin.

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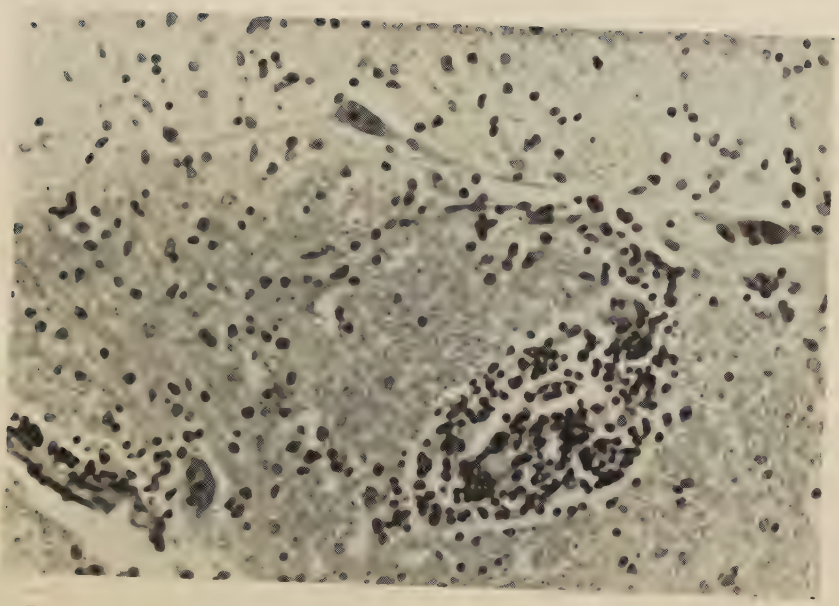

Fig. 1.—Medulla Case 4. Vascular and perivascular infiltration. (H. & E. × 200.)

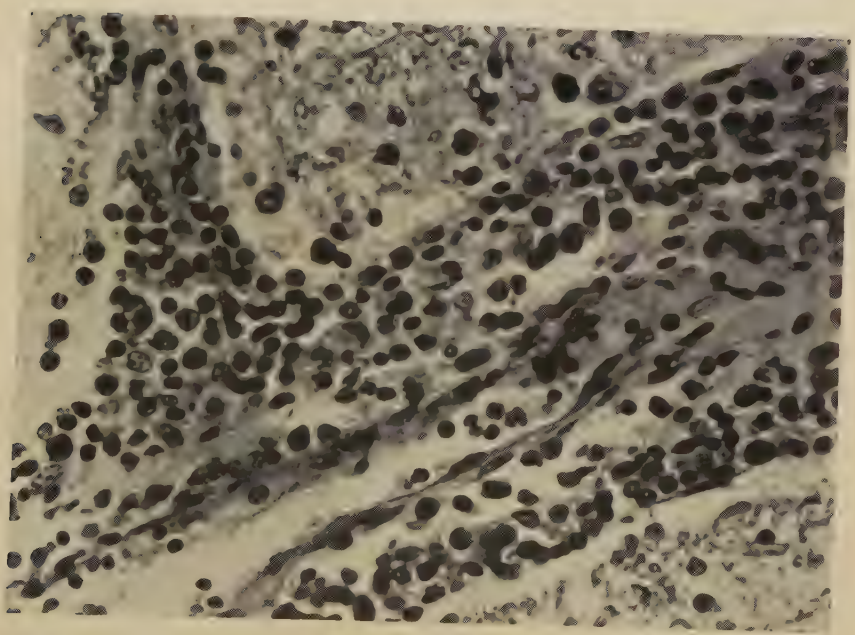

Fig. 2.—Medulla Case 4. Lymphocytes, larger mononuclear cells and occasional plasma cells closely packed round a small vessel. (Smith & Quigley × 540.)

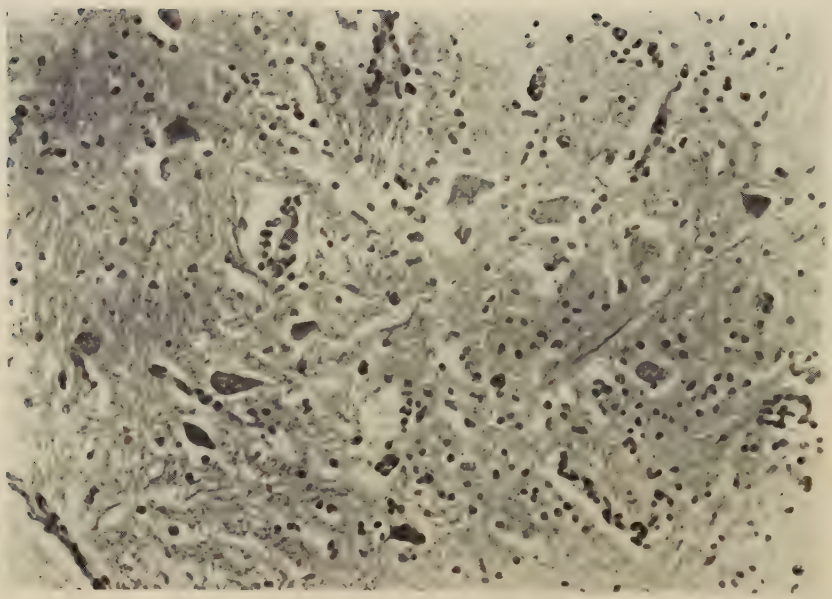

FIG. 3.—Medulla Case 1. Degenerating nerve cells and cell infiltration into the grey matter. (H. & E. × 190.)

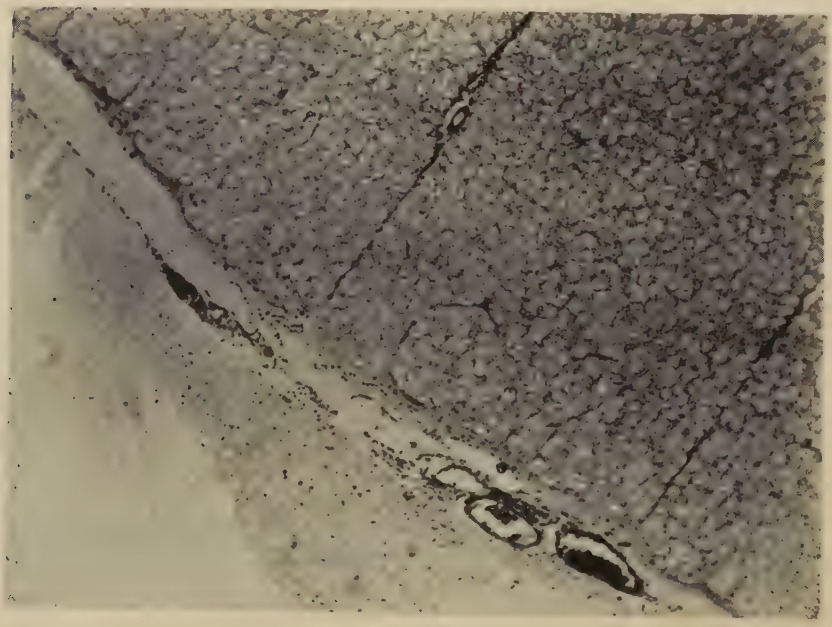

FIG. 4.—Thoracic cord Case 5. Vascular infiltration of two small vessels. Small foci of infiltrating cells in the white matter. Slight meningeal reaction. (H. & E. × 45.)

GLUCOSE TOLERANCE IN THE BOVINE

By

F. R. Bell and E. R. Jones

Veterinary Research Laboratory, Entebbe, Uganda

INTRODUCTION

Glucose tolerance has been investigated in several species of domestic animals, but only two references could be traced dealing with this subject in the bovine. We have been unable to verify the statement of Whitnah, Riddell and Hodgson (1933) quoted by Link (1940) that an appreciable increase of blood sugar follows the oral administration of glucose, thus confirming the work of Kennedy *et al.* (1939) quoted by Dukes (1942) which shows that little change occurs in the blood sugar of mature cattle after feeding glucose up to 1·75 gm./kg. body weight. Preliminary work carried out both with glucose and cane sugar by drench, in doses up to 8 gm./kg., did not produce variations of blood sugar outside the range of normal daily fluctuations, and for this reason the detailed data have not been included in this paper.

The peculiar anatomical structure of the adult bovine stomach probably accounts for the low blood sugar response to oral glucose administration, since the glucose after passing to the rumen, becomes diluted and is only slowly passed to the small intestine from where it is absorbed. Dukes (1942) suggestion that part of the glucose in the rumen may be fermented to non-carbohydrate substances, may have some bearing on this point.

Subcutaneous injection of glucose produced a satisfactory rise in blood sugar, but the results obtained by this method (see below) suggested a trial of the intravenous route of administration, thereby overcoming any errors due to absorption.

EXPERIMENTAL

Ten laboratory-bred adult bovines (cross-bred Ankole-Zebu), three to four years old, were selected after preliminary clinical and blood examination. Physical and chemical findings (see Table I) all came within the range given by Jones (1943) for Ankole and Zebu stock. Regular clinical and blood examination during the course of the tests showed no abnormalities with the exception of bovine 4974, which developed an *A. marginale* infection, and on which no further tests were performed.

Intravenous injection was carried out with sterile 50 per cent. solution of anhydrous glucose in distilled water by means of a canula, and the rate of administration controlled by a suitable burette and stopcocks. After preliminary trials it was decided that a uniform rate of injection of 20 c.c. per minute was most convenient, and only tests carried out at this rate are included in this paper. The blood sample before injection was obtained with the canula, while

TABLE I

BLOOD COMPOSITION OF TEN ADULT BOVINES USED FOR INTRAVENOUS GLUCOSE TOLERANCE TESTS

No.	*Sex*	*Erythrocytes (Millions)*	*Leucocytes*	*% Cell Volume*	*C.I.*	*Hb. Gms. %*	*Plasma CO_2-C.P. (Vols. %)*	*Mg./100 ml. Blood*				*Mg./100 ml. Serum Ca.*	*Ca/P Ratio*
								Glucose		*NaCl*	*In.P.*		
								Mean	*Range*				
4973	♂	7·6	8,830	36	1·16	11·6	66·4	56·0	48·4/67·3	445·5	4·6	10·9	2·37
4974	♂	6·7	9,000	34	1·27	11·2	48·5*	50·0	44·1/60·6	495·0*	3·4*	9·7*	2·85*
4980	♂	7·4	13,570	34	1·12	10·9	66·4	58·8	53·0/63·1	470·3	4·5	11·0	2·45
4983	♀	7·7	8,550	33	1·12	11·3	60·7	50·7	38·8/60·4	495·0	4·2	10·0	2·38
4987	♀	7·4	7,800	37	1·31	12·8	64·5	55·7	48·2/61·0	445·5	4·1	9·4	2·29
4988	♀	7·3	6,720	33	1·15	11·1	62·4	51·1	47·9/53·0	462·0	6·1	10·7	1·76
4991	♂	7·1	10,650	37	1·32	12·3	61·4	56·4	47·6/72·2	445·5	4·4	9·8	2·22
5000	♀	8·7	5,940	36	1·02	11·7	64·5	46·1	37·1/51·9	462·0	3·0	10·3	3·43
5011	♀	5·0	6,280	31	1·46	9·6	62·6	52·8	46·8/63·3	462·0	3·2	10·0	3·13
5012	♀	9·0	9,440	35	0·99	11·7	74·0	57·6	47·3/78·7	453·8	4·6	11·2	2·43
Extreme range ...		5·0/9·0†	5,940/13,570†	31/37†	0·99/1·46†	9·6/12·8†	60·7/74·0	—	37·1/78·7	445·5/495·0	3·0/6·1	9·4/11·2	1·76/3·43
Mean		7·4	8,678	34·6	1·19	11·4	64·8	53·3	—	460·2	4·3	10·4	2·5
(Number of Estimations)		(35)	(35)	(35)	(35)	(35)	(9)	(88)	(88)	(9)	(9)	(9)	(9)

NOTES.—* Values obtained after finding *A. marginale* in blood ; not included in Mean and Extreme Range.
C.I. = Colour Index calculated on formula 7·6 × Hb. (gms./100 ml.) ÷ R.B.C.s (in 0·1 mill.) using Jones (1943) standards as normals. Haemoglobin = 11·8 gms./100 ml. ; erythrocytes = 9·0 million per cu.mm.
† Calculated from individual means.

subsequent samples were drawn from the jugular vein by needle at intervals of 2, 15, 30, 60 and 120 minutes after the completion of the injection.

The methods used for physical and chemical blood analysis were the same as those previously recorded by Jones (1943), using oxalated blood throughout. To assess the reliability of the original Folin and Wu (1920) technique, control determinations were carried out on aqueous solutions of pure dextrose (" Analar ") preserved with toluol, the results of which are shown in Fig. 1, from which it can be seen that the observed values did not show as great differences from the theoretical as those recorded by Oser and Karr (1926). For this reason all blood sugar values recorded in this paper are the actual uncorrected Folin-Wu results obtained.

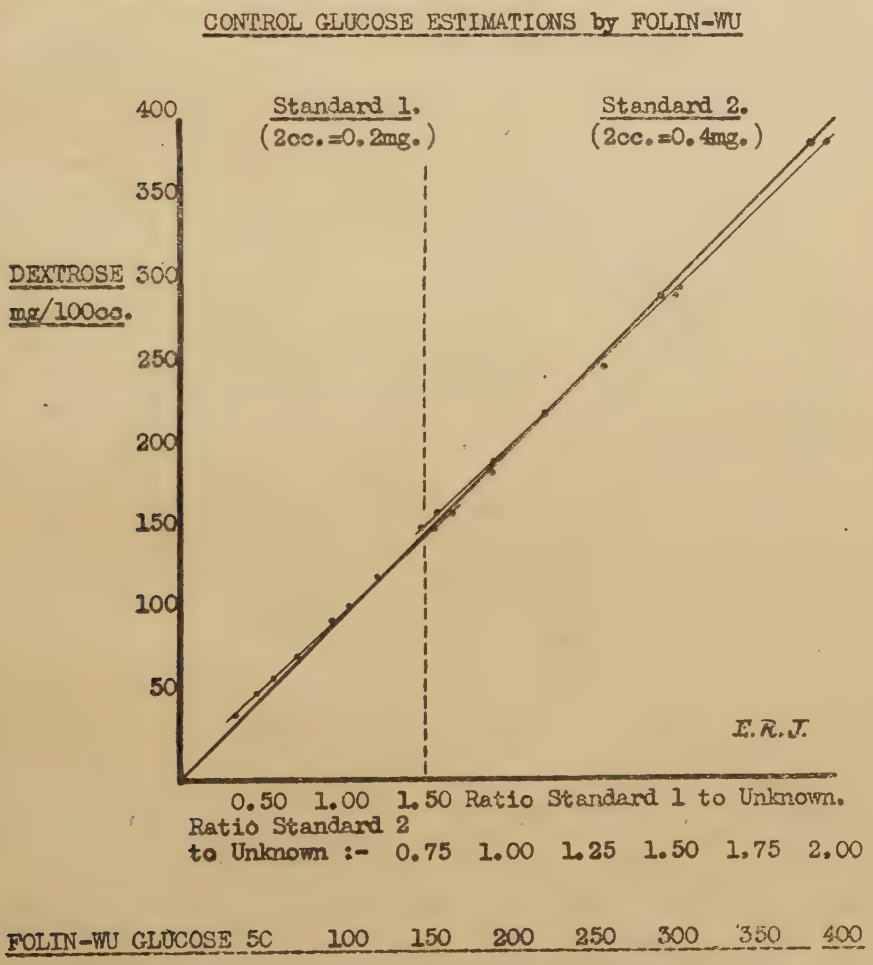

FIG. 1.—Graph showing mean values obtained by the original Folin-Wu method in 65 control estimations on aqueous solutions of pure dextrose preserved with toluol.

Urine was not collected by catheter as it proved extremely simple to obtain samples as desired by stimulation of the clitoris and prepuce. Estimation of glycuresis was made in all cases by the standard Benedict qualitative technique, using 5 c.c. Benedict's reagent + 8 drops of urine and heating in a boiling water bath for five minutes. In view of the statement by Boddie (1933) that Nylander's reagent was much more reliable than either Benedict's or Fehling's, a number of comparative tests were made prior to the experiments under review. It was shown that Benedict's test was by far the most reliable and sensitive (even to 0·1 per cent. glucose) and never gave a single false positive. Boddie also states in his evaluation of the urinary constituents of lactating and pregnant cows that 58 per cent. show sugar in the urine. It must be noted that all our animals were either males or non-gravid heifers, but they never showed glycosuria except after the administration of glucose. The estimations of glucose in the urine were determined by the standard Benedict quantitative method.

DISCUSSION

The blood sugar response to glucose administration by the oral, subcutaneous and intravenous routes are illustrated in Fig. 2. From the example given of oral administration it is apparent that no significant change occurs in the blood sugar, the values all being within the normal range. In the case of subcutaneous administration, a significant hyperglycaemia was obtained after one hour, but absorption appeared to be slow since the blood sugar had only fallen slightly two hours after the injection. On the other hand, intravenous glucose administration produced a marked hyperglycaemia with a return to normal within two hours in all cases. For this reason the intravenous route was selected as the most suitable for glucose tolerance tests in the bovine.

The hyperglycaemia produced by the intravenous injections was dependent on the dosage of glucose administered, giving mean values of 118·4 mg./100 ml. in the 0·1 gm./kg. dose, 168·6 mg./100 ml. in the 0·2 gm./kg. dose, and 253·5 mg./100 ml. in the 0·5 gm./kg. dose. The curves obtained in the intravenous tests (Fig. 2) at these doses show that the blood sugar returned to normal in the majority of cases within one hour, and in all cases within two hours, irrespective of the dosage of glucose. The maximum glycosuria produced was 5·7 per cent. sugar, and the maximum duration of glycosuria was 94 minutes, after which period negative samples were obtained. Glycogenesis in the bovine therefore parallels that observed in other species, from which it can be assumed that the storage and regulating mechanism in the bovine is similar to that of other animals.

The figures obtained for the removal rate of glucose from the blood were calculated by the method of Hamilton and Stein (1942) from the slope of the logarithmic curve of the blood sugar values.

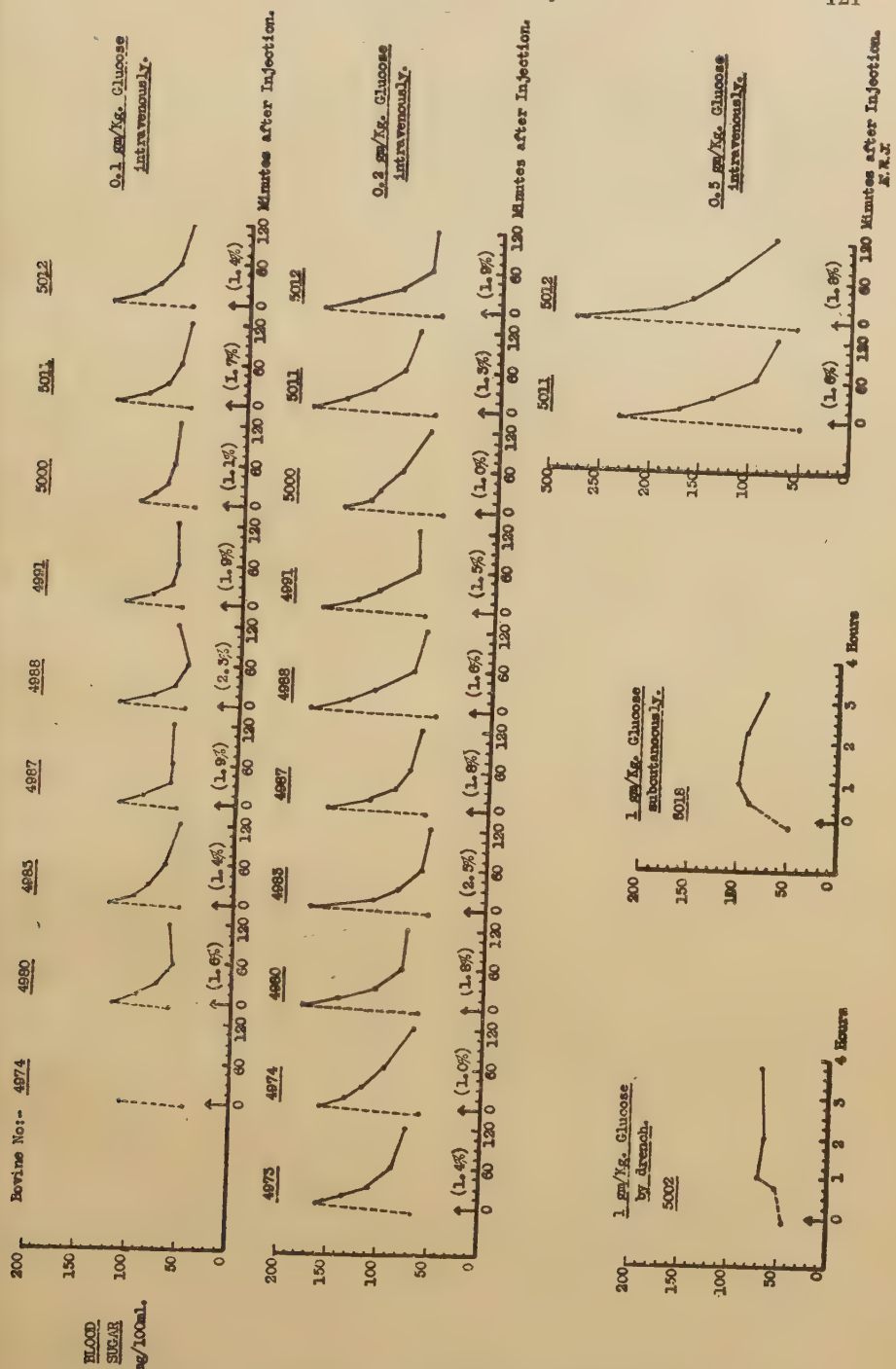

FIG. 2.—Blood-sugar curves obtained in 20 intravenous glucose tolerance tests. The arrow indicates the end of the injection period, whilst the figures in brackets show the removal rate of glucose from the blood, as measured from the slope of the logarithmic curve in each case. For comparison, the results obtained with oral and subcutaneous administration of glucose are also shown on the same scale.

The range of 1·0 to 2·5 per cent. per minute for the 20 intravenous tests in bovines is thus smaller than that noted by Hamilton and Stein in children (0·9 to 3·8 per cent.). The means obtained with 0·1, 0·2 and 0·5 gm./kg. of glucose are almost identical, thus showing a sugar removal rate independent of the degree of hyperglycaemia. It should, perhaps, be noted that these figures are not comparable with the slopes of the curves shown in Fig. 2, which are not plotted on logarithmic paper.

The increase in blood sugar following the intravenous injection of glucose in doses ranging from 0·03 to 0·5 gm./kg. body weight (Fig. 3) show that blood sugar responses proportional to the amount of glucose injected were obtained in all cases, but that no pronounced hyperglycaemia was produced until a dosage of 0·1 gm./kg. was reached, at which level the increase was approximately 100 per cent.

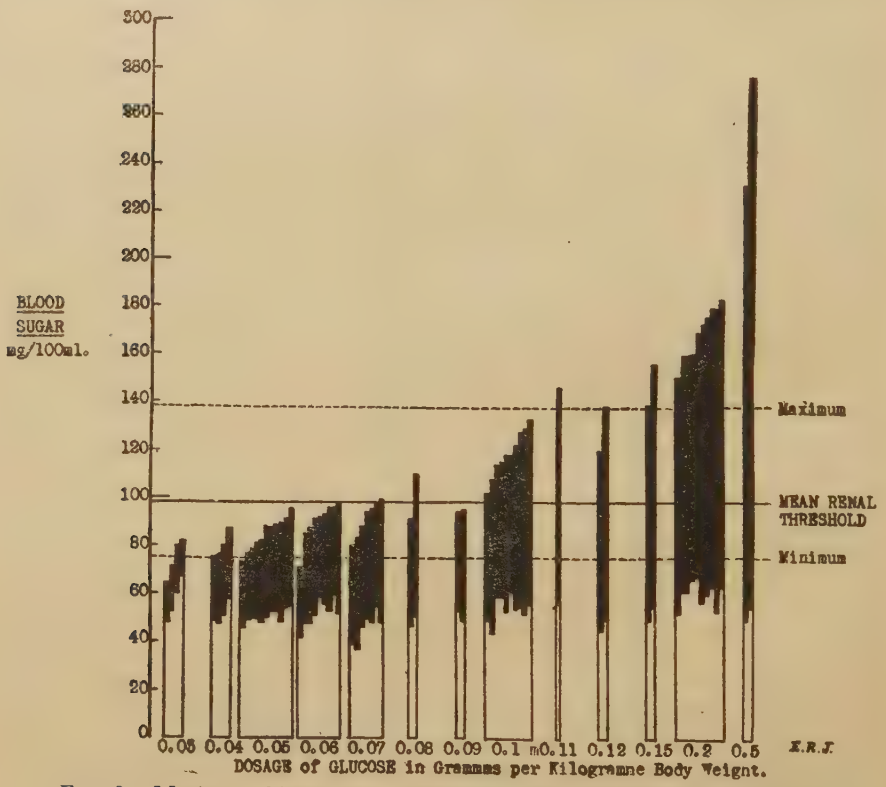

FIG. 3.—Maximum blood sugar response in ten adult bovines to intravenous glucose injection. The solid area shows the increase two minutes after injection.

In determining the ascending renal threshold a single blood sample was taken two minutes after the injection (in addition to the sample before injection) and the sugar value obtained was assumed

to be the maximum response. All available urine samples from the time of the injection were collected and tested for glycosuria, the specimens being obtained usually within six minutes after completion of the injection. The bladder was emptied in most cases prior to the injection of glucose. The criterion taken in determining the ascending renal threshold in each animal was the minimum blood sugar value corresponding to a glycosuria = + (Benedict's qualitative test). It will be seen from the results obtained that the limits of ascending renal threshold showed a somewhat wide variation, giving a mean value of 98. In six of the ten animals the threshold closely approximated the mean value, two were considerably lower and two considerably higher. Duplicate threshold determinations were made on the two animals showing low values and almost identical results were obtained. We are unable to account for these low and high threshold values, as these animals appeared clinically normal and their extreme values bear no relation to sex incidence.

In the case of the low thresholds, it is noteworthy that Bovine 5000 gave the lowest blood sugar response to glucose injection at all the doses it received, but on the other hand Bovine 5011 showed no evidence of abnormal response. Chemical blood findings showed no evidence of acidosis in these animals from their CO_2-C.P. values, but they showed the lowest In. P. values obtained in the ten bovines, and in consequence the highest Ca/P ratio. This evidence may possibly have some bearing on the low thresholds obtained, in view of the statement by Mattice (1936) that " diminution in the calcium-ion concentration of the blood lowers the renal threshold." The possibility remains, however, that such low thresholds may prove to be cases of renal glycosuria.

With regard to the high thresholds, Bovines 4974 and 4991 showed no abnormal response to glucose injection, and their chemical blood findings did not suggest any metabolic dysfunction.

Descending renal threshold values have been calculated from the blood sugar curves in Fig. 2, and the mean value of 102 obtained closely compares with the ascending mean value of 98. In the individual animals the descending and ascending values closely approximated. It appears, therefore, that the ratio of the mean renal threshold to the resting blood sugar value in the adult bovine is comparable to this ratio in other species.

From the data obtained, the opinion was formed that the maximum dosage of glucose that can be tolerated by intravenous injection without glycuresis is 0·05 gm./kg. in the adult bovine. A dosage of 0·2 gm./kg. is necessary to cover the entire range of renal threshold.

No untoward effects were noted after intravenous glucose injections in quantities up to 0·5 gm./kg. body weight, producing a maximum hyperglycaemia of 275·8 mg./100 ml. and glycosuria lasting almost two hours.

SUMMARY

1. The results of glucose tolerance tests on adult bovines by oral, subcutaneous and intravenous routes are compared.

2. Hyperglycaemia proportional to the dosage of glucose injected intravenously is recorded, with a maximum of 275·8 mg./100 ml. The blood sugar returned to normal within two hours in all cases.

3. Values for the removal rate of blood sugar after intravenous injection ranged from 1·0 to 2·5 per cent. per minute, and appeared to be independent of the degree of hyperglycaemia produced.

4. Renal threshold determinations showed an ascending blood sugar mean of 98 mg./100 ml., and a descending mean of 102 mg./100 ml.

5. The maximum glycosuria produced was 5·7 per cent. sugar, and the maximum duration of glycosuria 94 minutes.

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REFERENCES

Boddie, G. F. (1933). *Vet. Rec.*, **13** (N.S.), 1128.
Dukes, H. H. (1942). *The Physiology of Domestic Animals.* 5th Edition. New York: Comstock Publishing Company, Inc. p. 450.
Folin, O., and Wu, H. (1920). *J. biol. Chem.*, **41,** 367.
Hamilton, B., and Stein, A. F. (1942). *J. lab. and clin. Med.*, **27,** 491.
Jones, E. R. (1943). *Vet. Rec.*, **55,** 128.
Kennedy, W. L., and co-workers. (1939). *J. dairy Sci.*, **22,** 251.
Link, R. P. (1940). *J. Amer. vet. med. Ass.*, **97,** 261.
Mattice, M. R. (1936). *Chemical Procedures for Clinical Laboratories.* London: Henry Kimpton. p. 67.
Oser, B. L., and Karr, W. G. (1926). *J. biol. Chem.*, **67,** 319.
Whitnah, C. H., Riddell, W. H., and Hodgson, R. E. (1933). *J. dairy Sci.*, **16,** 347.

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STAPHYLOCOCCOSIS IN GEESE

By

W. T. Rowlands and H. Williams Smith,

School of Agriculture, University College of N. Wales, Bangor

INTRODUCTION

Losses associated with lameness among young geese seem to have received little attention in this country, but have been well recognised on the Continent for many years.

Lucet (1892) described an "infectious osteo-arthritis" of young geese in France, incriminating *Staphylococcus aureus* A similar condition was later recognised in geese and ducks in Germany by Freese (1907), who described acute and chronic forms, and by Loeffler (1910). Van Heelsbergen (1919) recorded the disease in Holland. Hutyra and Marek (1926) refer to the condition under the heading "Staphylomycosis," and Ward and Gallacher (1920) as "Osteo Arthritis of Young Geese and Ducks." Hole and Purchase (1933) reported a somewhat similar disease in pheasants in this country, and Jungherr (1933) a like condition in turkeys and later, Jungherr and Plastridge (1941), in fowl in America.

In all these cases a staphylococcus has been accepted as the aetiological factor.

The writers recently investigated losses in four widely separated outbreaks among young geese in N. Wales. The worst occurred in a flock of 125 newly introduced young geese. Advice was not sought until 15 had died rather suddenly with no symptoms of serious illness. Poisoning was at first suspected, but later disproved. The birds had been allowed a completely free range during the day and were shut up in an old byre bedded down on chaff at night. Further cases, some of which recovered, followed at irregular intervals over a period of four to six weeks, and about 30 died in all.

SYMPTOMS

The disease appeared in two forms, an *acute* form terminating fatally in two to three days, and a *chronic* form of two to three weeks' duration, followed by slow recovery or gradual relapse and death, due mostly to starvation and accident.

The *acute* form often showed no symptoms but was sometimes characterised by lameness in one or both legs, several joints (leg and wing) being slightly swollen, hot and painful. Affected geese moved about reluctantly with drooping wings. When recumbent, support was gained by extending the neck, resting the head on the ground, spreading the wings and turning the feet inwards. Diarrhoea was a constant symptom and temperatures were slightly elevated. The birds appeared fairly bright despite their condition.

The *chronic* form was characterised by extreme emaciation, anaemia and lameness with infection localised in one or more joints. Generally one joint only was affected, most commonly the hock or

tibio-metatarsal, although the stifle or hip joint was occasionally involved. Slight diarrhoea was present. Temperature was normal.

PATHOLOGY

A total of 19 cases was examined *post mortem*. A striking lesion in the majority of cases was the presence of small black scabs on the *underside* of the web. Some webs examined were covered with these scabs whilst in others only a few were present. These scabs were hard, dry and generally superficial. Others, however, containing necrotic material often extended through the epidermis, penetrating into the deeper layers.

In the *acute* cases several wing and leg joints were enlarged and inflamed. The synovial capsule was much distended with a thin, white, serous exudate which clotted on exposure to air. The synovial membranes and articular surface were acutely inflamed. The periarticular tissue and tendon sheaths were swollen, congested and distended with serous fluid. The intestine and liver were congested and a small excess of clear pericardial fluid was a constant feature. The bone marrow was soft in consistency and dark red and glistening in appearance.

In *chronic* cases the pathological state of affected joints had progressed to that of a typical chronic inflammation. A large amount of fibrous tissue had been laid down around the joint, causing great increase in size. Abscesses were commonly found around the joint and in the tendon sheaths, each taking the form of a yellow necrotic centre surrounded by a hyperaemic zone and much fibrous tissue. The synovial capsule was much thickened, the inner surface being often necrotic. Inspissated serum or fibrin replaced the fluid seen in joints in the acute cases. The articular surfaces in some cases were completely necrosed and crumbled away from the bone on being touched. The necrosis in other cases was confined to small areas, giving to the articular surface a pitted appearance. The muscles of the affected limbs were markedly atrophied and the whole carcase was emaciated and anaemic. A little pericardial fluid was again noted while a chronic nephritis with a deposition of urates was a common feature.

BACTERIOLOGY

Primary cultures from affected joints, fluids, heart blood, bone marrow and visceral organs were made in 10 per cent. sheep blood agar and nutrient broth.

In *acute* cases, pure cultures of a typical staphylococcus were obtained from heart blood, spleen, liver, bone marrow and all lesions. In *chronic* cases pure cultures of a similar organism were obtained from lesions only. Gram-stained smears prepared from joint fluids in acute cases revealed Gram-positive rounded cocci, and on histo-

logical examinations of the scab lesions similar organisms were seen in the depth of the lesion.

Although the four outbreaks were widely separated, a remarkable consistency was noted in the character of all the strains of organisms isolated with but few minor exceptions, suggesting strongly that the staphylococcus so isolated had some degree of specificity.

Morphology.—A typical strongly Gram-positive, non-motile coccus arranged in clumps.

Metabolism.—Agar: (18 hours). Colonies opaque, glossy, tacky, and convex, 1·5 to 2 mm. diam. *Shake agar*: Growth only on surface. *McConkey*: Abundant growth. *Anaerobic media*: No growth. *Blood agar*: All strains, with one exception, were non-haemolytic on 10 per cent. sheep blood agar after incubation as long as six days at 37° C., and finally standing at room temperature. The exception gave a fairly wide zone of clear haemolysis in blood agar after incubation for 24 hours. *All* strains were non-haemolytic on hen and goose blood agar, but produced a very narrow zone of haemolysis on rabbit and guinea-pig blood agar. *Broth and Peptone water*: Growth occurred as a uniform turbidity.

Biochemical.—All strains produced acid but no gas in mannite, lactose, trehalose, glucose, maltose and saccharose (in three days). None fermented salicin, raffinose, dulcite, xylose or inulin. Gelatin was readily liquefied; indole was negative (peptone water four days). Litmus milk showed acid with slight clot.

Toxin Production.—No toxin was produced as judged by an intradermal and i/v inoculation in rabbits and mice.

Haemolysin Production.—No strains produced a filtrable haemolysin.

Pigment Production.—With the exception of one strain, all produced a golden pigment which in two cases changed later to yellow. The exception produced a pure white pigment after 24 hours' incubation at 37° C. and later standing at room temperature. (This strain was cultured from the only carcase received from one particular outbreak, but apart from this characteristic showed no other differences from the other strains.)

Coagulase Production.—All strains were coagulase positive when tested by the tube and rapid slide test. Fibrolysin was demonstrated by the method of Christie and Wilson (1941).

Pathogenicity.—Unfortunately, no opportunity was offered to study the pathogenicity of the organism in geese or fowl. Intravenous injection into mice and subcutaneous inoculation of guinea-pigs and rabbits produced no ill-effect.

SEROLOGY

As losses were high it was decided to examine the blood of the geese in the affected flocks for antibody content. Agglutination tests were decided upon, since in the absence of any demonstrable filtrable haemolysin no anti-haemolysin would be present.

Antigen.—Two antigens, using two strains from different outbreaks and a third prepared from a staphylococcus isolated from a case of bovine mastitis, were used. The organisms were grown on agar plates for three days, washed off with normal saline, centrifuged to remove pigment and standardised to the density of a No. 6 Brown tube.

Sera, including geese and bovine were set up in normal saline in falling dilutions to volumes of 0·5 c.c. An equal volume of antigen was added, giving final dilutions from 1 : 10 to 1 : 640. Tubes were incubated at 37° C. for 24 hours and examined.

All geese sera agglutinated the antigens prepared from the two specific goose strains to equal titre but the bovine strain was never agglutinated beyond a titre of 1 : 10. Bovine sera gave no agglutination in any dilution with either the goose or bovine antigen. The titre of all geese affected with the disease in its chronic form was never less than 1 : 160, usually 1 : 320 and occasionally 1 : 640. The reactions given by the geese showing no symptoms in the affected flocks was very variable, a factor depending undoubtedly on the extent to which they had been exposed to the infection. Several agglutinated to a titre of 1 : 80 but never higher, while the majority agglutinated at 1 : 10 or 1 : 20.

Since the flocks in question were considered to be heavily infected a few normal goose bloods obtained elsewhere were examined but rarely agglutinated to more than 1 : 10, usually not at all.

TREATMENT

Biotherapy.—It was decided to investigate the possible therapeutic and prophylactic value of an autogenous vaccine (formalinised whole culture). For this purpose half the flock was inoculated and the other half left untreated as controls. Since it was considered likely that practically all the geese had been exposed by then to infection, the work was commenced with little real hope of success.

The vaccine was prepared by growing the staphylococcus isolated from the particular flock on soft agar for four days, washing off with normal saline, expressing the juice from the agar and adding 0·25 per cent. of formalin, standardising to Tube No. 6 (Brown) and incubating for three days. After testing for sterility the vaccine was used in a dose of 1·0 c.c. inoculated subcutaneously.

Following inoculation, no further deaths occurred in the controls, but several of the chronically affected geese which had been injected died within the following few days. From this the authors conclude that vaccination caused a flare up from the chronic condition. After further blood tests it was interesting to note that vaccination had no effect at all on the agglutinating activity of the serum.

Chemotherapy.—The effect of sulphadiazine on the viability of the staphylococcus isolated was investigated by means of the " ditch-plate " culture method. Evidence obtained suggested that it had no effect, but by a similar test crude penicillin proved bacteriostatic.

DISCUSSION

The disease described is regarded as being probably identical with that occurring on the Continent. Since in the acute form of the disease, death is frequently sudden and unheralded by symptoms such as those of arthritis, it is considered a little misleading to refer

to it merely as a staphylococcal arthritis. The term " staphylococcosis " as used by others in similar connections is, therefore, advanced to meet this contingency. The term " staphylococcosis of geese " is tentatively proposed to describe the condition, since in its acute phase it develops a septicaemic character masked in its occurrence by the localised chronic form which is probably the commoner. In view of its possible high mortality rate (20 to 25 per cent.), it is surprising that no previous record has been made of its occurrence in this country.

A knowledge of a parallel condition in lambs (tick pyaemia) suggests that it is an inoculation infection. It is often assumed that the causal organism is merely a skin inhabitant which has assumed a pathogenic role on passing the epidermal barrier. In the disease investigated, much thought has been given to the manner in which infection gained entrance and it is tentatively concluded that this occurred through wounds on the webs caused by some sharp material, possibly plants, *e.g.*, sedge, of which there was an abundance and in which nesting was common in the flock most seriously affected. The " scabs " on the web certainly appeared to be the oldest and probably the primary lesions. Jungherr (1941) regarded the insertion of an antipeck device to be the portal of entry in poultry. Hole and Purchase (1931) refer to infection of wounds following nesting in thistles. Now the opportunity for such wounding among geese flocks is vast and if, therefore, the staphylococcus isolated is merely a skin inhabitant it seems all the more remarkable that such outbreaks of staphylococcosis in geese are not of more frequent occurrence. Hence it is concluded that either some hitherto unrecognised secondary factor is involved, permitting a previously facultative saprophyte to become pathogenic or that the staphylococcus concerned is indeed a specific pathogen in this disease.

Assuming the organism to be a specific pathogen, the question arises as to how the strain made its appearance among these flocks. Enquiry showed that in the largest flock all the birds were recently purchased from two different sources, while in each of the others individual birds had recently entered the affected flocks from outside. This suggested the possibility of " carrier " birds—a possibility which seems reasonable in the light of the result of the blood tests made, indicating that many apparently normal birds in an infected flock may have specific agglutinins and perhaps harbour infection. The contamination of nesting places or edges of drinking pools might well lead to subsequent infection. If this theory of the carrier bird can eventually be accepted it appears likely that we have in the blood test a ready means whereby to detect them.

Considerable interest is attached to the staphylococcus held responsible for the condition. It has been designated pathogenic on the basis of its ability to produce coagulase and in this sense is in keeping with the pathogenic strains isolated from various avian sources by Jungherr (1941). The strain of staphylococcus isolated

in this work did not, however, as in the case of Jungherr's strains, produce a dermo-toxin or filtrable haemolysin capable of detection by the method employed.

The position of this staphylococcus in relation to the pathogenicity of the group generally, also bears examination and lends support to the modern concept of a true pathogenic staphylococcus. Haemolysis on blood agar can no longer be accepted as the sole criterion, and the signal importance of the coagulase test is emphasised.

As far as the work has gone it has shown that the blood of normal geese does not contain any specific antibody whereas infection with this staphylococcus soon stimulates its production. Such agglutinating serum, however, appears to be somewhat type specific, since it fails to agglutinate a pathogenic staphylococcus of bovine origin. This again suggests a certain specificity in the case of the staphylococcus isolated.

Treatment of staphylococcosis in geese is, at the moment, unsuccessful. Vaccination in the authors' hands was a failure in a therapeutic sense, since it appeared only to precipitate a fatal issue. The use of such treatment was also reported as without effect by Jungherr (1933). Similarly, treatment with sulphonamides proved valueless in the hands of Jungherr and Plastridge (1941). Whether or not at some future date penicillin might prove beneficial and economical remains to be seen.

SUMMARY

A disease of young geese termed "Staphylococcosis," which appears in N. Wales, is described and is regarded as probably identical with that reported as occurring on the Continent.

It assumes an *acute* form with death in two to three days, but more commonly a *chronic* form with recovery or subsequent relapse and death.

The acute form is septicaemic in character, with some localisation of infection in several limb joints, mainly of the legs. Diarrhoea is a constant feature. In the chronic form infection is localised to the joints. There is marked emaciation, lameness and diarrhoea.

The pathology is fully described and emphasis is placed on the presence of black scabs on the under surface of the web.

It is thought that these constitute the primary lesions and the portal of entry of infection.

The staphylococcus isolated is regarded as pathogenic on the basis of the coagulase test, and as having some degree of specificity. It produces no haemolysis on 10 per cent. sheep, hen and goose blood agar, no filtrable haemolysin and no demonstrable toxin.

Autogenous vaccination was valueless prophylactically and therapeutically. On the contrary, it appeared to precipitate an early and fatal termination of chronic cases.

Sulphadiazine was without bacteriostatic effect *in vitro*, but penicillin was definitely bacteriostatic.

REFERENCES

Lucet. (1892). *Ann. Inst. Past.*, **6**, 162.
Freese. (1907). *Deut. tierärztl. Wschr.*, **15**, 322.
Loeffler, F. (1910). *Arch. Tierheilk. Suppl.*, **26**, 289.
Van Heelsbergen, T. (1919). *Cent. Bakt., Ref.*, **68**, 272.
Hole, N., and Purchase, H. S. (1931). *J. comp. Path.*, **44**, 251.
Jungherr, E. (1933). *J. Amer. vet. med. Ass.*, **35**, 243.
Jungherr, E., and Plastridge, W. N. (1941). *Ibid.*, **98**, 321.
Christie, R., and Wilson, H. (1941). *Aust. J. exp. Biol.*, **19**, 329.

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INFECTION OF THE BOVINE LUNGS WITH AN ORGANISM RESEMBLING *BACILLUS ACTINOIDES*

By

F. Blakemore

Institute of Animal Pathology, University of Cambridge

INTRODUCTION

Theobald Smith (1918, 1921) demonstrated the presence of a pleomorphic organism in the lungs of calves affected with bronchopneumonia which he designated *Bacillus actinoides*. Cultures were obtained with some difficulty from infected material and only under a reduced oxygen tension. In fluid media growth took the form of small flakes and pear-shaped bodies up to 10μ in diameter were observed on the surface of the colonies. Morphologically, the organism showed marked pleomorphism, young cultures containing bacillary and bipolar forms, while in older growths these were replaced by ring-like structures and granules.

The classification of *B. actinoides* has not been altogether satisfactory. Topley and Wilson (1936) refer to it as *Actinobacillus actinoides*. Nelson (1931, 1933) compared its morphological, serological and growth requirements with strains of an organism recovered from rats, which was undoubtedly *Streptobacillus moniliformis*, and concluded that they were probably varieties of the same species. Dienes and Edsall (1937) and Klieneberger (1938) also commented on this similarity, but while there seems little doubt that there exists a close relationship between them, *B. actinoides* differs from *S. moniliformis* in certain of its morphological features, its microaerophilic requirements and its inability to infect rodents.

S. moniliformis has been recovered from Haverhill fever, an infection of human beings characterised by erythema and arthritis, and various conditions in rodents, such as pneumonia, middle ear disease and cervical abscesses. There is also evidence that it is a common inhabitant of the pharynx of rats (Strangeways, 1933). Early cultures show great pleomorphism with bacilli and filaments which stain unevenly, and there are many swollen degenerate forms. Occasional filaments develop spherical or oval, club-shaped moniliform swellings, either sub-terminally or along their length. After 24 to 48 hours' incubation many of the filaments are replaced by chains of cocci, globules and ring-like forms. Primary isolation is difficult except in rich serum media; the organism is non-motile and Gram-negative.

Considerable interest has been taken in *S. moniliformis* since Klieneberger (1935) showed that a second filtrable microbe could be obtained from cultures which differed from the parent growth in that it did not produce bacillary forms but resembled the organism of bovine pleuropneumonia and consisted of small granules, filamentous structures and bodies of varied appearance. It was designated L1 and Klieneberger believes that such organisms exist as symbionts in cultures of Streptobacillus, being responsible for certain of its morphological characters. The work of Dienes (1939), Heilman (1941) and Wilson Smith (1941) did not support this view, and they concluded that the L1 organism was a variant. As stated by Dienes,

this interpretation does not lessen the importance of the original observations since, if it is a variant, further study may give some information on filtrable forms of bacteria.

During the course of an investigation into pneumonia of bovines an aerobic organism which in many respects resembled *B. actinoides*, was isolated from cases of the disease. Considerable difficulty was experienced in obtaining pure cultures because of its delicate habit of growth but it was possible to recover strains from two outbreaks. An account will be given of the organism concerned, together with a description of the disease with which it was associated. Because of the resemblance of the organism to *S. moniliformis* observations were also made to ascertain whether an associated filtrable form was present in the cultures.

SOURCE OF MATERIAL AND ISOLATION OF THE ORGANISM

Case 1.—The disease occurred in the early autumn in a group of fifteen 6- to 12-month-old heifers; the majority was affected and the mortality was 25 per cent. The symptoms, which were those of coughing with signs of pneumonia in severe cases, were believed to be due to parasitic bronchitis but the usual treatment by intratracheal injections aggravated the condition. *Post-mortem* examination of one typical case killed *in extremis* revealed bronchopneumonia affecting almost the whole of the anterior and cardiac lobes. A careful search failed to reveal *Dictyocaulus viviparus*. The bronchi contained an abundant muco-purulent discharge and the cut surface of the lung showed a greyish white infiltration around the bronchi so that they stood out prominently against the intervening hepatised lung. The interstitial tissue was not involved to any great extent; suppurative foci were not observed and there was no pleurisy. The thoracic lymphatic glands did not show any significant change. In sections of the lungs it was found that there was degeneration of the bronchial epithelium and the exudate contained numerous polymorphs. Peribronchial infiltrations, consisting principally of lymphocytes and commencing fibrosis with thickening of the walls of the bronchi, were the most characteristic feature of the lesions. The alveoli in the pneumonic areas showed an indistinct architecture and contained serous exudate, leucocytes and erythrocytes.

Smears of the bronchial exudate stained by Giemsa revealed a large number of pleomorphic bacilli which did not grow on nutrient agar or in broth containing 5 per cent. ox serum. Cultures were, however, obtained in 30 per cent. ox serum broth, growth appearing in 48 hours as a flocculo-membranous deposit at the bottom of the tubes.

Case 2.—There was no connection between this outbreak and the previous one. It occurred during the autumn and affected cattle which were 6 to 12 months old. Again parasitic bronchitis was suspected and the symptoms were similar to those observed in the

first case. The distribution and appearance of lesions were also essentially the same but in the two animals on which an autopsy was made the lesions extended to the anterior parts of the diaphragmatic lobes and there were abscesses of varying size throughout the affected lung. There was no evidence that the lesions resulted from parasitic infestation of the bronchi.

Although the organism to be described could be readily demonstrated in smears of the bronchial exudate its isolation proved difficult, but it was finally recovered in pure culture from one of the specimens by plating on chocolate agar containing 30 per cent. of ox serum. The lungs of both animals were also infected with *Corynebacterium pyogenes* and alpha streptococci; these organisms may have been responsible for the suppurative lesions.

MORPHOLOGY AND CULTURAL CHARACTERS

In morphological studies of *S. moniliformis* and the associated L organisms, special methods have been employed because of the difficulties encountered in preparing suitable stained smear preparations. These have included dark ground illumination and the staining of colonies by the agar fixation method (Klieneberger, 1934). In the present investigation, though the organism stained poorly and unevenly, smears and impression preparations of colonies stained by Giemsa (Gurrs, R66) were found to be suitable. Colonies prepared by the agar fixation method were also examined, as were dark ground preparations, but they did not yield any further information regarding the morphology of the organism.

In the bronchial exudate smear preparations revealed a high degree of pleomorphism and the strains from both outbreaks were identical in this and other respects. Long and short bacilli were present, some of which stained intensely, while others were swollen, pale and degenerate. Bipolar, comma and vibrionic forms were observed and some of the bacilli had granules arranged along their length. In addition to the bacillary types, cocci lying singly and in short chains were abundant. Some of the elements in the original cultures were Gram-positive but later the organism became frankly Gram-negative. It was non-motile and capsules were not observed.

While at first cultures were obtained only in media enriched with blood or 30 per cent. ox serum, after prolonged subcultivation broth containing 5 per cent. was found to be suitable. Haemolysin was not produced. The organism was aerobic and growth was not improved either by sealing the tubes or admitting CO_2. Cultures remained viable for five days and the organism could not be maintained by the usual method of drying *in vacuo*.

In liquid media growth took the form either of small compact granules which tended to settle to the bottom of the tube or as a flocculo-membranous deposit with some general turbidity. Cultures at 24 hours were composed of uniform bacillary forms 2 to 3μ long which stained evenly. On further incubation pleomorphism was

marked and the forms previously referred to were apparent, but after three to four days the bacilli disappeared and were replaced by cocci 1μ in diameter, ring-like structures and bacterial debris.

The formation of cocci was one of the most striking features of the organism and they appeared to arise from the bacilli since they were found along the length of many which were degenerate. That they represented a resting stage in the development of the organism was evident from the fact that growths could readily be obtained from cultures in this phase.

On blood agar minute translucent colonies appeared after 24 hours, which increased in size with further incubation to 2 to 3 mm. and became opaque and yellowish brown. By plating broth cultures it was occasionally possible to identify two types of colony after 24 to 48 hours' incubation. Both were translucent and of the same general appearance, but while one variety was only just visible to the naked eye the other was 0·5 to 1 mm. in diameter. The larger colonies were composed of short deeply staining bacilli and short filaments 10 to 15μ long which did not show branching, while the small ones consisted of cocci, ring forms, faintly staining globular bodies 3 to 4μ in diameter and a few degenerate bacilli. Whereas broth cultures of these small colonies showed many bipolars and a few cocci during the early stages of growth, those from the larger colonies consisted almost entirely of uniformly stained bacilli. Further subcultures gave rise to a mixed type of growth and the differences observed appeared to represent only a temporary variation.

An examination of the surface growths and broth cultures failed to reveal the pear-shaped bodies which Theobald Smith (1921) observed in colonies of *B. actinoides*. It seems probable that these were identical to the empty globules observed in cultures of *S. moniliformis* which Partridge and Klieneberger (1941) found to contain a high percentage of cholesterol probably produced from the serum broth.

FILTRATION OF CULTURES

In view of the similarity of the organism to *S. moniliformis*, which it resembled morphologically, except that it did not show the same tendency to produce long filaments and moniliform swellings, an examination was made for the presence of an associated L organism. This was repeatedly attempted soon after isolation by the filtration of broth cultures through tested Berkefeld V filters. The results were consistently negative, and this observation was later confirmed by Miss Klieneberger to whom a strain was submitted.

TRANSMISSION EXPERIMENTS

Early cultures obtained from Case 1 were tested for pathogenicity on groups of six mice by intranasal injection under anaesthesia and by the intraperitoneal route. All the mice survived and no lesions

were found when they were killed after seven days. A further experiment was carried out in which mice to which cultures had been given intranasally were redosed by the same route with an equal amount of normal bovine serum after four days and again the result was negative.

The effect of the organism on month-old calves was determined using the second subcultures. One animal was injected subcutaneously with 5 c.c. of a broth culture and this treatment did not produce any general or local reaction; another was given 10 c.c. intranasally and housed with a normal calf of the same age. The inoculated animal developed a cough after seven days and symptoms of pneumonia followed. Its condition became progressively worse and it was seriously ill when killed on the 13th day. The temperature reaction was variable throughout the period but not significantly high and never exceeded 105° F. On autopsy the lesions were found to be confined to the thorax. There was an acute bronchopneumonia involving almost the whole of the anterior and cardiac lobes. The pericardium was distended with serous exudate but there was no pleurisy. The organism was recovered in pure culture from the affected lungs. Histological examinations revealed a bronchopneumonia characterised by marked peribronchitis as in the field condition. The in-contact calf remained normal for 28 days when it was noticed to be coughing. The condition became more severe but at no time did definite signs of pneumonia appear and it finally recovered after an illness of 22 days. The animal was kept under observation for a further 18 days and then killed. On *post-mortem* examination the right anterior lobe was found to be collapsed and fibrosed, but cultures failed to demonstrate the test organism or any other infection.

Another series of experiments was carried out after the organism had been subcultured at intervals of three days for a month. A calf was inoculated with 5 c.c. of culture subcutaneously. Two more were treated intranasally, one receiving 10 c.c. of whole culture and the other the same amount of a Berkefeld V culture filtrate. The results of this experiment were entirely negative and the calves were normal when killed a month later. At the same time an attempt was made to infect a sheep by the intratracheal injection of culture but the animal remained normal.

DISCUSSION

Very little is known regarding the primary bacterial causes of bronchopneumonia in the bovine and infection with the organisms commonly associated with the condition is usually believed to take place following damage by other agents such as parasites or some reduction in the general resistance of the animal. For instance, Pasteurella of low virulence and pyogenic cocci are often found in

bronchopneumonia, developing after exposure or transit in cold weather, and Mitchell (1939) described *C. pyogenes* infection in adult cattle associated with a heavy infestation with *Dictyocaulus viviparus*. Such evidence does not lessen the importance of these organisms once they have become established or mean that they may not occasionally be directly responsible for pneumonia, but it would seem desirable to enquire more fully into the aetiology of the condition as diagnosed in the field in order to classify the disease more fully and determine the factors influencing its development.

It has been suggested that virus infections may serve as an important predisposing cause and this aspect of the problem has not been sufficiently investigated. Lamont and Kerr (1939) found indications of virus infection in outbreaks of calf pneumonia in Northern Ireland and concluded that the condition was similar to swine influenza, but their evidence was not conclusive. They isolated an associated organism which it was not possible to maintain in culture but they believed that it belonged to the Haemophilus group because of its general morphology and the fact that enriched media was necessary for isolation. The disease was reproduced by the intranasal administration of suspensions of diseased lung and there was some evidence that the infective agent would pass through Seitz filters. The period of incubation was 5 to 14 days and, as in the case of the disease produced experimentally in this investigation, its onset was slow and it was afebrile. Virus pneumonia has also been described by Baker (1943) amongst calves in America, and Waldmann and Köbe (1935) have reported cases in Germany although the general importance of the observations made by the latter cannot as yet be assessed since the condition they described may be related to, if not identical with, epidemic coughing of horses which occurs there.

Apart from the possibility of virus infection being primarily responsible for some outbreaks of pneumonia in the young bovine the aetiological role of certain bacteria warrants further attention. In this respect the findings of Theobald Smith (1918) are of special interest since *C. pyogenes* and other organisms were often observed as secondary invaders in *B. actinoides* infection. Experimentally, he was unable to reproduce pneumonia with his cultures, but following intratracheal injections localised foci of necrosis were found in the lung, and it seems probable that this damage enabled saprophytic bacteria to produce further injury to the lung tissue. With the organism under consideration a pneumonia was produced by the use of cultures not long after isolation, but after maintaining it under artificial conditions for a month it was completely non-pathogenic. The question arises as to whether the alteration in this respect was due to an associated virus or symbiont in the early cultures. Attempts were made to obtain evidence of a second organism with negative results and although the presence of virus cannot altogether be excluded, the results would be more readily explainable on the

assumption that the organism lost its virulence when grown under artificial conditions.

The relationship of the disease to that described by Theobald Smith is a matter of some interest. In many respects the conditions are alike but the organism under consideration did not cause suppuration when injected subcutaneously, as was the case in Smith's experiments. Furthermore, whereas it was possible to set up a severe bronchopneumonia with cultures, Smith succeeded in producing only small areas of necrosis in the lung by intratracheal injection of *B. actinoides*. Unfortunately it was not possible to make an histological study of the early lesions in the disease under consideration, but focal necrosis was not a feature of advanced cases.

There is also a similarity between the organisms concerned. They both require enriched media since neither grow in the absence of serum or blood, but *B. actinoides* differs in its oxygen requirements. A culture of *B. actinoides* was not available for comparison, but the morphology of these organisms is essentially the same and of a kind which is so striking as to warrant them being classified together, especially as the grouping of bacteria of this description is based on their appearance and cultural characters. Their relationship to *S. moniliformis* is another matter for consideration since again there are many points of resemblance, but until further information is obtained it would seen advisable to regard them as distinct.

From the practical point of view, the observations indicate the desirability of further classifying the pneumonic conditions of bovines since errors in diagnosis may lead to incorrect treatments being applied, especially if bronchitis due to *D. viviparus* is mistaken for pneumonia of bacterial origin. Unfortunately, it has not been possible to determine the incidence of the disease investigated, but it seems possible that it has been overlooked owing to the difficulty of isolating the organism concerned. The diagnosis of two unconnected outbreaks suggests that it may not be uncommon.

SUMMARY

An organism which in many respects resembles *B. actinoïdes* was recovered from two outbreaks of bovine bronchopneumonia. The disease was reproduced experimentally in calves without difficulty by the intranasal injection of early cultures, but after further subcultivation the organism lost its pathogenicity. There was no evidence that the loss of virulence was due to the disappearance of an associated filtrable bacterium, and it is suggested that it arose as the result of some modification of the organism when grown under artificial conditions.

An account is given of the cultural characters of the organism as compared with *B. actinoides* and *S. moniliformis*.

REFERENCES

Baker, J. A. (1943). *J. exp. Med.*, **78,** 435.
Dienes, L., and Edsall, G. (1937). *Proc. Soc. exp. Biol., N.Y.*, **36,** 740.
Dienes, L. (1939). *J. infect. Dis.*, **65,** 24.
Heilman, F. R. (1941). *Ibid.*, **69,** 32.
Klieneberger, E. (1934). *J. Path. Bact.*, **39,** 409 ; (1935). *Ibid.*, **40,** 93 ; (1938). *J. Hyg., Camb.*, **38,** 458.
Lamont, H. G., and Kerr, W. R. (1939). *Vet. Rec.*, **51,** 673.
Mitchell, T. M. (1939). *Ibid.*, **51,** 666.
Nelson, J. B. (1931). *J. Bact.*, **21,** 183 ; (1933). *Ibid.*, **26,** 321.
Partridge, S. M., and Klieneberger, E. (1941). *J. Path. Bact.*, **52,** 219.
Smith, Theobald. (1918). *J. exp. Med.*, **28,** 333 ; (1921). *Ibid.*, **33,** 441.
Smith, Wilson. (1941). *J. Path. Bact.*, **53,** 29.
Strangeways, W. I. (1933). *Ibid.*, **37,** 45.
Topley, W. W. C., and Wilson, G. S. (1936). *The Principles of Bacteriology and Immunity*. London. p.1003.
Waldmann, O., and Köbe, K. (1935). *Berl. tierärztl. Wschr.*, **51,** 2.

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PHARMACOLOGICAL STUDIES ON THE UTERUS

By

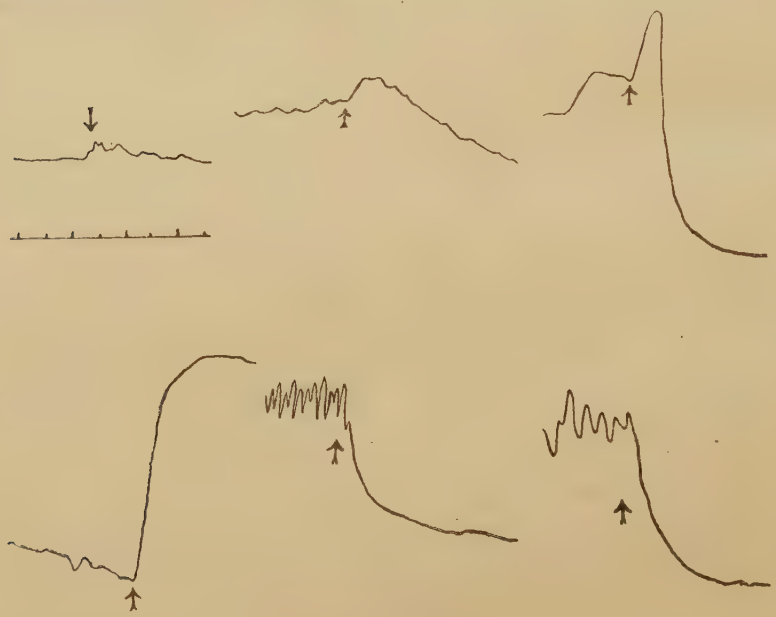
Frank Alexander,

From the Department of Pharmacology, University of Edinburgh.

INTRODUCTION

From a study of the action of various drugs on the uterus, information of therapeutic and physiological value may be obtained. The common laboratory animals and man have been studied extensively in this way, but little work has been done on the uteri of the larger domestic animals. The mare's uterus was investigated by Horvath (1937), who found that pituitary extract and carbachol (carbamyl choline) increased the rhythmic contractions of the uterus. Benesch and Steinmetzer (1931, 1932) recorded the movements of the cow's uterus by means of a balloon placed in the lumen; they found that pituitary extracts and quinine were powerful ecbolics, but ergot preparations were inactive; epidural anaesthesia did not inhibit the movements. Graf and Paschke (1932) observed a paralysis of the isolated bovine uterus by novocaine. Studying the cow's uterus by *in vivo* and *in vitro* techniques, Benesch and Steinmetzer (1936)

Fig. 1.—Action of adrenaline.



Concentrations—parts per million; N.P.—non-pregnant; Preg.—pregnant.

Fig. 2.—Action of Acetylcholine.

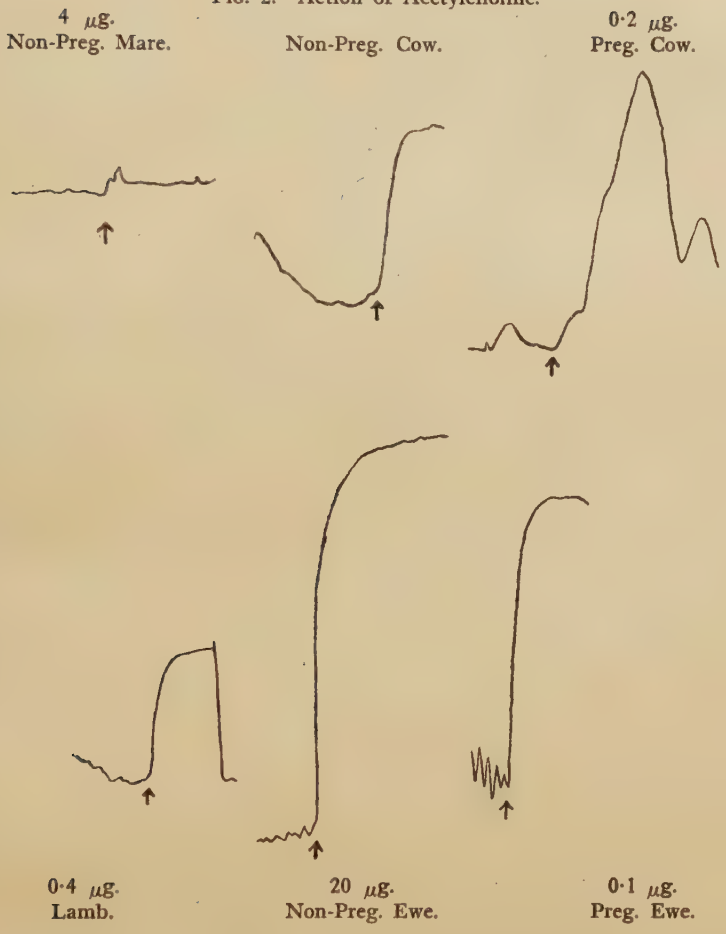
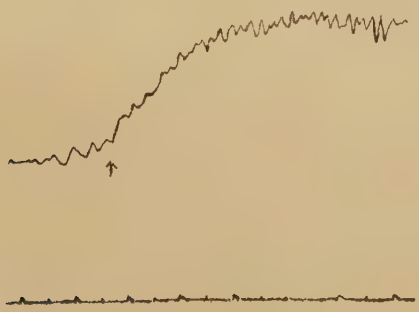


Fig. 3.—Action of ergometrine on the uterus of a pregnant ewe.



found that carbachol was a very active ecbolic. Gunn (1944) has investigated the effects of adrenaline, acetylcholine and various allied substances on the sheep's uterus.

The present paper is concerned with the actions of certain humoral and oxytocic substances on the uterus of sheep, cow and mare with a view to studying the innervation of this organ and ascertaining the value of certain ecbolics.

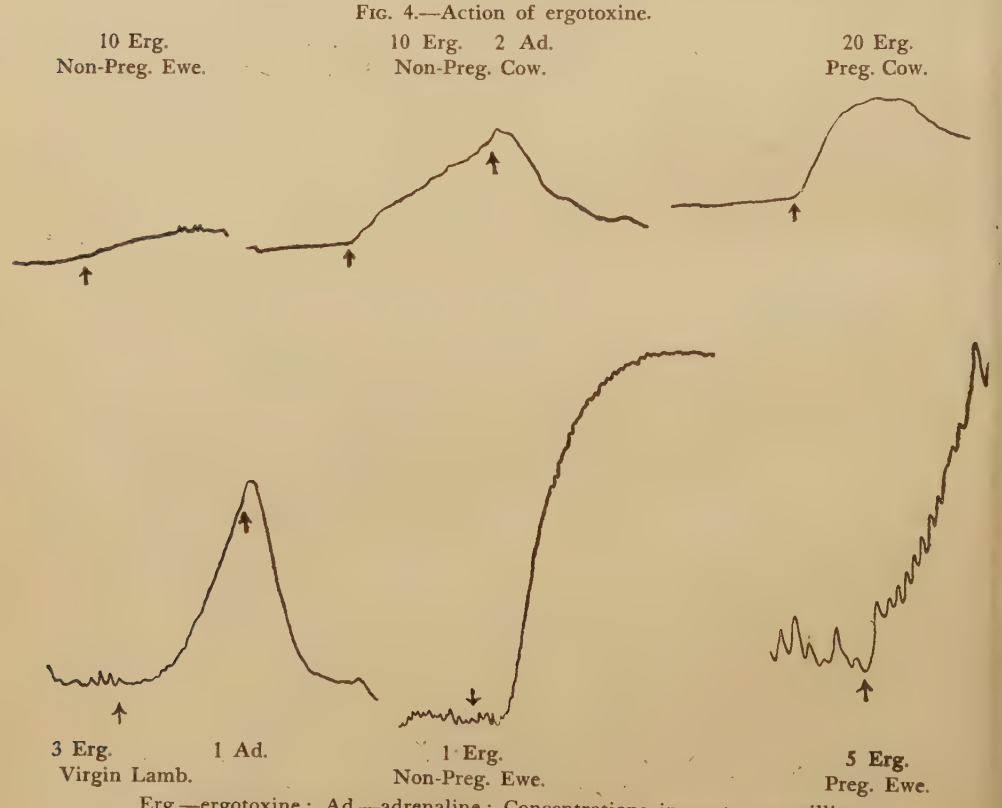
METHOD

The uteri were studied by the method of Kehrer (1907). Strips were cut from the wall of the organ and suspended in Dale's solution in a bath of 10 c.c. capacity which was replenished from a reservoir of 200 c.c. capacity. Both the bath and reservoir were carefully maintained at a temperature of 37° C. Solutions of various drugs were added to the bath and the effects recorded on a smoked drum. Adrenaline, acetylcholine, ergotoxine, posterior pituitary extract and ergometrine were investigated.

RESULTS

Adrenaline caused contraction of the virgin sheep's uterus in a concentration of 1/1,000,000 and a similar concentration of this drug

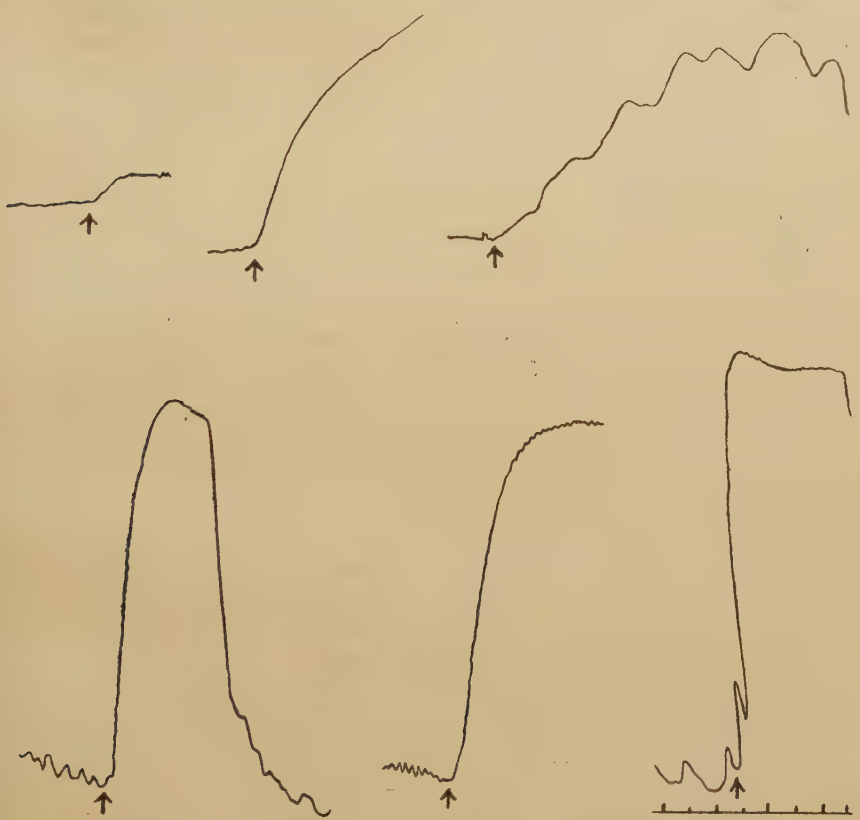
FIG. 4.—Action of ergotoxine.



Erg.—ergotoxine; Ad.—adrenaline; Concentrations in parts per million.

inhibited the non-pregnant and pregnant ewe uterus. One record was obtained of a contraction of the pregnant ewe uterus after the addition of adrenaline to the bath. In Gunn's (1944) experiments on sheep, he obtained similar results with virgin uterus and with pregnant uterus, but adrenaline caused a contraction of a non-pregnant parous uterus. No explanation can be suggested for the discrepancy between this result and that shown in Fig. 1; in both the pregnant and non-pregnant cow the uterus was inhibited by a concentration of 1/1,000,000 adrenaline. The non-pregnant uterus of the mare was contracted by a concentration of 1/1,000,000 adrenaline. In all the instances where adrenaline produced a motor effect, this was abolished by ergotoxine. The inhibitor effects were not so affected.

FIG. 5.—Action of posterior pituitary extract.



Acetylcholine produced a marked contraction of the uteri of all the animals studied. All these uteri were remarkably sensitive to this substance, the non-pregnant organ being more sensitive than the pregnant. The sheep's uterus usually responded to a concentration of 1/10,000,000, and although the other animals were less sensitive, a concentration of 1/500,000 was usually effective in producing a response.

The uteri of all the animals studied were contracted by ergotoxine in both pregnant and non-pregnant states. This effect was always produced by a concentration of 1/100,000. The effects of ergometrine on the isolated uteri of the laboratory animals and woman is feeble and in most cases absent (McLachlin, 1938). The uterus of the pregnant ewe was the only organ studied which gave a satisfactory response; a contraction was produced by a concentration of 1/100,000.

The uteri of sheep and cows, pregnant and non-pregnant, and that of the non-pregnant mare were contracted powerfully by posterior pituitary extract, a concentration of 0·001 unit/c.c. produced this effect in most cases.

The results described are illustrated by the following figures: Fig. 1 shows the effect of adrenaline on the various uteri investigated, Fig. 2 that of acetylcholine, Fig. 4 that of ergotoxine, Fig. 3 that of ergometrine on the pregnant sheep's uterus, and Fig. 5 that of posterior pituitary extract on the various uteri.

DISCUSSION

The value of studying the actions of a drug on the isolated uterus before conducting experiments *in vivo*, was clearly shown by Dale and Laidlaw (1912). The experiments described here suggested that ergotoxine and posterior pituitary extract would be effective ecbolics for sheep, cows and mares.

The evidence obtained from the actions of adrenaline and acetylcholine on the uterus is of value in determining the type of innervation possessed by this organ. The uterus is supplied by the hypogastric nerve which is essentially sympathetic, and there is some evidence of a parasympathetic supply through the pelvic nerve. (Gruber, 1933.) Since the humoral theory of the transmission of nerve impulse has become universally accepted (Loewi, 1935), pharmacological evidence on the innervation of an organ has an increased importance.

It has been shown by various writers (see Reynolds, 1939, and Labate, 1941), that stimulation of the hypogastric nerve produces the same effect on the uterus as the intravenous injection of adrenaline, and the effects of adrenaline on the uterus *in situ* were paralleled by its effects on the isolated organ (Gunn and Gunn, 1914, and Ogata, 1921). In view of the findings of these workers it seems reasonable to suppose that the action of adrenaline on the isolated uterus of the sheep, cow and mare would produce the same effect as stimulation of the hypogastric nerve. The experimental results

showed that this action varied with the species and with the state of the uterus. The uteri of the virgin sheep and the non-pregnant mare were stimulated, whereas those of the non-pregnant parous sheep, pregnant sheep, pregnant and non-pregnant cow were inhibited.

The fact that acetylcholine has a powerful stimulant action on all the uteri on which it has been tested, suggests that these uteri may be supplied with nerves liberating acetylcholine (cholinergic nerves). These might come either from the pelvic nerves, or from the hypogastric nerves. The pelvic nerves are parasympathetic, and therefore likely to be cholinergic, but they supply very few fibres to the uterus. The hypogastric nerves presumably contain adrenergic fibres which liberate adrenaline or some similar substance, but there is evidence that they also contain cholinergic fibres running to the uterus in the dog (Sherif, 1935). In the absence of direct evidence, argument by analogy suggests the presence of cholinergic fibres in the hypogastric nerves of the sheep, cow and mare.

SUMMARY

The isolated uteri of sheep, cows and mares were studied pharmacologically.

Adrenaline in a concentration of 1/1,000,000 contracted the uteri of virgin sheep and non-pregnant mare, inhibited those of the pregnant and non-pregnant sheep, pregnant and non-pregnant cow.

Acetylcholine contracted the uteri of all the animals studied in all their various states.

Ergotoxine contracted all the uteri studied in a concentration of 1/100,000.

The uterus of the pregnant ewe was contracted by 1/100,000 ergometrine.

The uteri investigated in all their various states were contracted by posterior pituitary extract, 0·001 unit per c.c.

REFERENCES

Benesch, F., and Steinmetzer, K. (1931). *Wien. tierärzt. Mschr.*, **18,** 165, 193, 577, 615; (1932). *Ibid.*, **19,** 65; (1936). *Ibid.*, **23,** 225.
Dale, H. H., and Laidlaw, P. P. (1912). *J. Physiol.*, **45,** 1.
Graf, H., and Paschke, H. (1932). *Arch. wiss. prakt. tierhlk.*, **65,** 285.
Gruber, C. M. (1933). *Physiol. Rev.*, **13,** 497.
Gunn, J. A. (1944). *J. Physiol.*, **103,** 290.
Gunn, J. A., and Gunn, J. W. C. (1914). *J. Pharmacol.*, **5,** 527.
Horvath, E. (1937). *Wien. tierärzt. Mschr.*, **24,** 138.
Kehrer, E. (1907). *Arch. Gynaek.*, **81,** 160.
Labate, J. S. (1941). *J. Pharmacol.*, **72,** 370.
Loewi, O. (1935). *Proc. roy. Soc. B.*, **118,** 299.
McLachlin, A. D. (1938). *J. Pharmacol.*, **64,** 243.
Ogata, S. (1921). *Ibid.*, **18,** 185.
Reynolds, S. R. M. (1939). *Physiology of the Uterus.* Hoeber.
Sherif, M. A. F. (1935). *J. Physiol.*, **85,** 298.

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STUDIES ON THE HAEMATOLOGY OF SHEEP
IV. ERYTHROCYTIC AND THROMBOCYTIC PICTURES, AND VARIATIONS IN PHYSICAL ATTRIBUTES

By

H. H. Holman,

Animal Diseases Research Association, Moredun Institute, Edinburgh

INTRODUCTION

Very little detailed work has been carried out on the erythrocytic picture of sheep other than observations made on post-haemorrhagic and haemolytic anaemia, and this work has been referred to where appropriate. The wide range that must be considered normal—due, at least in part, to unrecognised worm infestation—again produces difficulty. Nevertheless, it has been considered desirable to attempt to set standards for variations *below the normal*, that is, Anaemia ; and *above the normal*, that is, Polycythaemia rubra, but in view of the tentative nature of this work, discussion on aetiology has been limited to a minimum.

In the case of thrombocytes the only important change that has been recorded is the diversity of shape occurring in haemolytic anaemia recorded by Chamberlin (1933). Only a few isolated observations on the various physical attributes occur in the literature and no statements as to their value have been made.

The normal range and the daily variations have already been dealt with (Holman, 1944a, 1944b, 1944c), but for convenience normal means for various constituents have been included in Tables V and VII.

ANAEMIA

Anaemia signifies a lack of blood and is a symptom rather than a disease. In man it has been classified by two methods, one dependent on cause, the other on effect. The first method, in which classification is based on aetiology, is advocated by Vaughan (1936), who recognises three main classes ; dyshaemopoietic anaemias due to failure or abnormality in blood production ; post-haemorrhagic following haemorrhage ; and haemolytic due to blood destruction within the body. The second method, in which classification is dependent on the alteration produced on the blood indices, was introduced by Wintrobe (1942), who formulated four main classes, namely, macrocytic, normocytic, simple microcytic and hypochromic microcytic. In man, where cell size plays such an important part in diagnosis, Wintrobe's classification is of great value, but in sheep where anaemia is mainly normocytic in type, the aetiological classification is to be preferred, and Vaughan's classification has been used in this article.

Post-haemorrhagic Anaemia

Figures for a case of acute post-haemorrhagic anaemia produced by bleeding a sheep from the jugular vein, are recorded in Table I and, for comparison, results obtained by other workers are summarised in Table II. Holman and Pattison (1941) found that in lambs this type of anaemia could be produced, or accentuated, by the rapid healthy growth following the sudden removal of heavy worm infestations that had previously delayed their development.

Blood Loss and Regeneration.—It has been shown that a sheep can be killed by the removal of 3·2 litres of blood over a period of 66 hours (Table I). On the other hand, a sheep can endure such a loss if spread over a period of two weeks (Table II). Stippled cells and polychromatic cells were observed as early as 48 hours after the first bleeding but were not numerous. It can be seen from Table II that these are the common regenerative changes that occur. They are not, however, limited to the low blood counts recorded in Table II, and where bleeding is slight but continual these changes may be observed where the count is as high as 6·0 million; once stimulated by a low count they may persist and be present in blood samples giving red counts of 7·0 or even 9·5 millions. Jolly bodies are uncommon but my records include one case in which anisocytosis and the presence of these cells were the only abnormalities observed.

Reticulocytes were not recorded in this investigation, as it was found that polychromasia or punctate basophilia was a more sensitive guide to regeneration than the occurrence of reticulocytes in films stained by the dry film method, and that, with the wet film method, figures for reticulocytes increased minute by minute for several minutes, rendering this method time consuming and impractical for field work. In the rabbit, Whitby and Britton (1939) found that the sum of the polychromatic cells and the stippled cells were equal in number to the reticulocytes. There is no reason to believe that this is otherwise in the sheep, for Fourie observed that all polychromatic cells were also reticulocytes and Fraser showed a close correlation between the numbers of stippled cells and reticulocytes. It appears feasible, therefore, that had the figures for polychromatic cells been recorded, these would have supplied the deficiency.

Cell Size and Shape.—In sheep, one of the first changes apparent in post-haemorrhagic anaemia is anisocytosis, but in blood films from healthy sheep there is normally an irregularity in size. Abnormality is therefore largely dependent on personal opinion and should be diagnosed with caution unless indicated by an alteration in the Price-Jones curve. Such curves for the sheep detailed in Table I are given in Table III.

When, in anisocytosis, macrocytes and microcytes balance each other in volume the mean corpuscular volume (M.C.V.) remains the same and the anaemia is still termed normocytic. This was the case in Table I and also in Andrew's sheep (Table II).

Whitby and Britton (1939) and Wintrobe (1942) record that in

TABLE I
FIGURES FOR BLED SHEEP

Hours	Blood withdrawn Lit.	R.C.	Hb.	P.C.V.	M.C.V.	M.C.H.C.	Sed.	Viscosity	Fragility	W.C.	Band	Poly.	Lym.	Mon.	Eos.	Bas.
0	0	12·0	12·6	32	26·7	39	3	7·0	0·5	5·5	0	17	74	3	5	1
18	0·5	10·2	10·5	26	26·4	41	4	5·9	0·5	7·2	4	27	64	3	3	1
24	1·0	9·0	8·6	21	23·4	41	5	4·2	0·5	6·9	7	22	67	2	0	0
42	1·5	6·3	6·8	18	29·0	38	10	3·3	0·5	8·3	6	23	70	1	0	0
48	2·5	*4·5	3·6	12	26·7	29	21	2·1	0·5	3·3	7	40	50	3	0	0
66	2·75	*3·4	3·4	9	26·7	38	21	2·6	0·5	7·5	15	40	40	5	0	0
67	3·2	Dead														

* Stippled cells and polychromatic cells present

TABLE II
ONSET AND SEVERITY OF REGENERATIVE CHANGES FOLLOWING BLOOD LOSS IN SHEEP

Reference: Author	Loss of Blood: Date	Litres	Period of time	Red count (mil.)	Regenerative Changes: P.B.	P.	J.B.	Ret.	Remarks
Holman	1944b	0·05	24 hrs.	n.c.	—	—	—		20 sheep. n.c. = no change.
Hollaender and Pellathy† ...	1928	13·3	38 mths	n.c.	—	—	—	—	n.c. = no change.
Wirth	1938				+++	+++	(+)	+++	Half total blood removed.
			Days						
Fourie	1931	2·1	4	5·6	—	—	—		M.C.V. 34.
		2·5	6	4·6	—	—	—		,, 39. Megalocytes observed.
		3·3	11	3·4	++	++	—		,, 45. Anisocytosis.
		4·0	14	3·7	++	++	—	+++	,, 45. No change in M.C.H.C.
Andrew	1942	1·0	4	7·2				1·5*	
		1·5	6	4·0				4·1*	
		2·5	11	3·3				10·0*	
		3·5	14	2·2				7·5*	No changes in M.C.V. or M.C.H.C.
Fraser	1930	Worm	May 9th	3·3	6·2*			7·4*	
		infest.	May 15th	2·5	15·0*			16·0*	Severe worm infestation.
			May 23rd	1·7	18·5*			26·0*	

(+) scarce.
++ 3 to 10 cells per field.
+++ many.

P.B. Punctate basophilia.
P. Polychromasia.
J.B. Jolly bodies.

Ret. Reticulocytes.
* Per cent.
† Quoted from Wirth (1931)

man if the stimulation of the bone marrow has been marked the anaemia may become macrocytic for a period of several days, and such a phenomenon is apparent in Fourie's sheep (Table II). Further, assuming *Haemonchus contortus* infestation to cause an uncomplicated post-haemorrhagic anaemia, Fourie demonstrated a slight irregular increase in cell size in three out of five cases, with one case showing a definite macrocytic anaemia with an M.C.V. of 50 and later 70 c.micra. Holman and Pattison, investigating a mixed worm infestation in which *H. contortus* was predominant, found a macrocytic anaemia in four out of ten cases, with M.C.V.s of 37, 41, 41, and 43 c.micra. In these cases blood films did not show a " shift to the right " in the Price-Jones curves and therefore it was assumed that the increased cell volume must have been due to a spherocytosis. Poikilocytosis was not evident in the sheep used for Table I or in the sheep bled by Fourie; it was, however, evident in some of the sheep he infected with *H. contortus*.

Table III
Price-Jones Curves for Bled Sheep Detailed in Table I

	Micra.							
Time. Hrs.	3	3·5	4	4·5	5	5·5	6	6·5
0	—	15	100	274	100	11	—	
42	7	34	170	203	65	20	1	
66	6	71	134	166	77	35	10	1

Haemoglobin Concentration.—In acute post-haemorrhagic anaemia the blood remains normochromic. This is the case in Table I and also in the examples given by Andrews and Fourie; the periods over which these sheep were bled did not exceed a fortnight. In chronic post-haemorrhagic anaemia the corpuscles become hypochromic and figures given by Fourie for three sheep infested with *H. contortus* for periods of over three weeks show this defect.

Physical Properties.—In Table I the figures for sedimentation give a logarithmic curve and those for viscosity a simple linear regression when the erythrocyte counts are taken as the independent variate. Fourie gives viscosity figures for one case of infestation and there, too, the figures are compatible with a straight line regression. Fragility remained constant.

Leucocytes and Thrombocytes.—In contrast to man, no leucocytosis was evident in the bled sheep detailed in Table I or in those bled by Fourie and Andrews. In Table I severe anaemia was accompanied by a slight neutrophilic reaction and the disappearance of eosinophils, and Andrews confirms this to some extent by giving a higher average for neutrophils and a lower one for eosinophils when comparing his bled sheep to controls.

No investigator has included a platelet count but in the case of the sheep detailed in Table I abnormal platelets—as described by Chamberlin (1933) in haemolytic anaemia—were seen, these included platelets as large as small erythrocytes, platelets with tails at one or both ends, and others round, sausage, or triangular in shape.

Haemolytic Anaemia

Selected details from accounts of haemolytic anaemia in sheep have been summarised in Table IV. It should be borne in mind, however, that some apparent differences are due to the amount of the noxa administered: thus in arsenical poisoning the dose was small and therefore produced mild regenerative changes, while with Type A toxin the dose was large and was lethal before regenerative changes were pronounced.

Haemolytic anaemia is in many respects similar to post-haemorrhagic anaemia and will not be treated in detail. The main difference lies in the liberation of blood pigment, evident either as icterus or haemoglobinuria, and an alteration in erythrocytic fragility.

TABLE IV
HAEMOLYTIC ANAEMIAS

	Enzootic toxaemic Jaundice	*Cl. Welchii Type A. toxin*	*Arsenical Poisoning*
Authors	*Chamberlin* (1933)	*Gordon* et al. (1940)	*Holman* (*unpublished*)
Neutrophilic reaction	Severe	Agranulocytosis	Mild
Regenerative changes	Marked, Normoblasts and Megaloblasts present	Present before 48 hours	P. +. P.B. +. after a few days with red count as high as 6·8 mil.
Indices	Sometimes achromasia	M.C.H.C. increased, possibly because free Hb. in blood plasma. M. C. V. unchanged	
Platelets—			
Increase in number	+	+	—
Diversity of shape	+	—	—
Haemoglobinuria	+	+	—
Icterus	+	R.B.C.s haemolysed in 7·5 per cent. NaCl.	—

Dyshaemopoietic Anaemias

In the present state of knowledge any attempt to classify the dyshaemopoietic anaemias in sheep must necessarily be both sketchy and tentative. Various sets of circumstances associated with this

type of anaemia are given in Table V, Part I, and, in contrast, some conditions in which pining or emaciation exist unaccompanied by anaemia are given in Part II.

From Table V, Part I, the existence of four types of dyshaemopoietic anaemia have been inferred. Firstly, a simple oligocythaemia in the case of inanition in a flock of sheep bearing a heavy worm burden; secondly, a hypochromic anaemia apparent in some cases of intestinal worms or of Johne's disease; thirdly, a macrocytic anaemia affecting pregnant ewes in certain districts of England, reported by Innes and Shearer (1940); and, fourthly, an aplastic anaemia seen in worm infested lambs severely affected with nodular necrosis.

TABLE V
MEAN FIGURES FOR ERYTHROCYTES IN PINING CONDITIONS

	Sheep	*R.B.C.s*	*M.C.V.*	*M.C.H.C.*
Dyshaemopoietic Anaemias				
Inanition and heavy worm burden	12	9·0	26	43
Intestinal worms (helminthiasis)	14	9·2	26	34
Johne's disease	9	8·6	27	35
Nodular necrosis	2	4·5†	29	36
Hyperchromic anaemia of pregnant ewes*	97	6·2	59	N
Pining unaccompanied by anaemia				
Scrapie	16	11·5	25	41
Solway pine‡...	21	10·3	28	40
Cobalt pine‡	10	11·6	26	42
Normal	100	11·5	27	41

* Innes and Shearer (1940).
N = Normochromic — absolute figures for Hb. not given.
† Without regenerative changes.
‡ Stewart and Holman (1944).

It must be emphasised that this classification is very general in its character and easily criticised. For example, only some worm-infested sheep showed hypochromia, while the presence of *H. contortus* in infestations must have complicated the picture by the production of some degree of post-haemorrhagic anaemia; the anaemia in Johne's disease may be partly or wholly due to the presence of intestinal worms; and, finally, a sheep affected with diarrhoea may suffer from a slight anhydraemia through the loss of water from the bowel, and thus an oligocythaemia may become obliterated, and the figure for the red count then recorded may be a high value in the normal range.

Another difficulty exists in the fact that owing to the wide normal range the figures for individual animals may fall at a low value within range, and to show abnormality it is necessary to take the average

for a group of such animals and show that this differs significantly from the average of the normal range. Thus we are in the unfortunate position of having to designate some groups of animals each of which has a low normal count as abnormal, a decision that involves either an arbitrary judgment or the use of a statistical test.

Oligocythaemia. Simple Anaemia.—In this type of anaemia there is a fall in the erythrocyte count without alteration in cell size or shape, or the appearance of immature cells. The figures given in Table V were obtained from a group of 12 hill sheep in apparent good health but showing worm egg counts of some 1,000 per g. These sheep were housed in loose boxes and refused to feed. The erythrocyte counts averaged eleven million on the first day but had fallen to 9·7 four days later. At the end of a week the count was 9·0 million, at this time the sheep were learning to take food from the trough ; a count made four days later was also 9·0 million.

This type of anaemia was also noticed in sheep at pasture during the months of February, March and April (Holman, 1944a) and it was pointed out that it persisted until the growth of fresh grass at the end of spring.

Hypochromic Anaemia.—This term refers to the combination of oligocythaemia and hypochromia ; the presence of an oligocythaemia distinguishes it from Chlorosis (Beattie and Dickson, 1943). It is regarded as occurring where there is interference with the anabolism of iron into haemoglobin, a cycle that requires the absorption of iron, copper and various food factors. In man this type of anaemia is usually microcytic but this does not appear to be the case in sheep. Groen (1940) states that where there is iron deficiency the resulting anaemia is rarely due to that cause alone and is usually the effect of a combination of several causes in which he includes the following : lack of iron, lack of stomach acid, a rapid period of growth, interference with absorption from the gut, and acute or chronic haemorrhage. He does not, however, distinguish between a normochromic oligocythaemia and a hypochromia but claims that either one or the other may be caused by iron deficiency.

Holman and Pattison noticed a hypochromic anaemia in rapidly growing lambs which were at pasture during the month of November. Those previously treated for worms showed an average M.C.H.C. of 32 per cent. and those untreated showed an average of 30 per cent. Hypochromia has also been noted in individual sheep suffering from intestinal worms or from infection with Johne's disease, and this may be due either to the interference with absorption from the gut, or, perhaps, where infestation with *H. contortus* is present, to chronic haemorrhage in which the iron reserves have been expended. Regenerative changes are apparent in some sheep where oligocythaemia is pronounced.

Hyperchromic Anaemia of Pregnant Ewes.—Innes and Shearer have shown that anaemia exists in ewes before and shortly after lambing. The districts in which this type of anaemia was apparent

included Leicestershire, Derbyshire and Cambridgeshire. Erythrocyte counts were often as low as 7·0 million and sometimes regenerative changes were observed. There was no hypochromia and the weighted mean for 97 ewes from four different sources gave a mean corpuscular volume of 59 c.micra. as compared to 26 c.micra for control ewes. The anaemia is thus classified as hyperchromic or normochromic and macrocytic in type. The ewes in other respects appear to be in good health.

Before leaving this type of anaemia it is important to note that although the M.C.V. showed a large increase in cell size, Innes and Shearer could not demonstrate a significant increase in the Price-Jones curve*. This is in agreement with the findings of Holman and Pattison, when investigating the macrocytic anaemia present with a severe infestation of *H. contortus*. These two isolated observations strongly suggest that macrocytic anaemia in sheep constitutes an increase in depth of the corpuscles without alteration of the corpuscular diameter. Thus there is a point of similarity between macrocytic anaemia in sheep and the anaemia observed in acholuric jaundice in man.

Aplastic Anaemia.—This is distinguished by a low erythrocyte count, without any visible sign of regeneration; that is to say, the red cells may remain normocytic and normochromic and there is no shift to the left as indicated by the appearance of immature corpuscles such as stippled cells or polychromatic cells. The point at which oligocythaemia ends and aplastic anaemia begins in the sheep is difficult to place.

Wirth (1938) studied regenerative changes in healthy sheep bled to half their normal count and on this basis perhaps it could be said that sheep with red counts as low as 5·5 mil. failing to show regenerative changes during succeeding days were probably suffering from aplastic anaemia.

Using this definition, cases of aplastic anaemia have been seen in lambs affected with nodular necrosis of the liver, in which oligocythaemia was progressive and fell as low as 4·0 mil., myeloid aplasia being indicated by an absence of regenerative changes and a diminution of the shift to the left seen in myeloid leucocytes. In this case aplastic anaemia was secondary to a hypochromic anaemia for both sheep had a haemoglobin concentration that was low in the normal range.

POLYCYTHAEMIA

With the term polycythaemia the qualifying adjective "rubra" is understood, hence the term signifies an excess of erythrocytes. Polycythaemia has not been recorded as a primary disease in sheep

* In the diagram given by Innes and Shearer the terms "Normal ewe" and "'Anaemic' ewe" were used to distinguish the two curves. Dr. Innes informs me that each was a composite curve built from the measurements of several thousand corpuscles on smears taken from a number of ewes. The statement made in Part 1 of this work, to the effect that a Price-Jones curve was obtained for "a pregnant ewe" is, therefore, inaccurate. (*J. comp. Path.*, 1944, **54**, 30.)

but secondary polycythaemia, or erythrocytosis, has been reported by Fourie as occurring in some cases of *H. contortus* infestation. It was apparent as an increase in the numbers of erythrocytes present for periods up to one fortnight after infestation, and was accepted as being due to the stimulation of haemopoiesis by a continuous haemorrhage.

Relative polycythaemia, or anhydraemia, denotes an apparent increase in red count due to an actual loss of water from the blood. It occurs in sheep affected by the action of *Cl. welchii* type D toxin (Gordon *et al.*) and may be the cause of an occasional high count of, say, 13 or 15 million, in lambs scouring as an effect of worm infestation.

STANDARDS FOR ERYTHROCYTES

The difficulty of fixing standards for a population suffering from differing degrees of sub-clinical worm infestation was commented upon when discussing standards for leucocytes (Holman, 1944c). All that need be said here is that, in the case of the erythrocytic measurements, there is not the same wide variation as seen in leucocytes, and therefore the estimation of normality and abnormality based on the standard deviation is more reliable. Such estimates for individual animals are given in Table VI, in which abnormalities in erythrocytes are classified as mild, moderate and severe. In addition to these standards, the M.A.D. (95 per cent.) (Holman, 1944b) can be used to detect small changes in experimental animals.

Table VI
Variations in Erythrocytes

	Mild	*Moderate*
Oligocythaemia. (Erythrocytes 10^6 per c.mm.)	8 to 6	6 to 4
Polycythaemia. (Erythrocytes 10^6 per c.mm.)	15 to 17	17 to 19
Macrocytic anaemia. M.C.V. c. micra	38 to 48	48 to 60
Hypochromic anaemia. M.C.H.C. per cent.	33 to 28	28 to 24
Anaemia if not judged by oligocythaemia		
Haemoglobin : g. per 100 ml.	9 to 7	7 to 5
Haematocrit : per cent.	23 to 17	17 to 12

THROMBOCYTIC PICTURE

The simple method of counting platelets that was used in this investigation was too unreliable an estimate to be a sensitive guide to small changes, but some increase in numbers was recorded in haemolytic anaemia, confirming Chamberlin's findings in haemolytic anaemia in Australia. An increase in post-haemorrhagic anaemia has not yet been recorded. No cases of thrombocytopenia have been reported in the sheep and the only important change in platelets would appear to be the change in shape observed by Chamberlin

(as given under " post-haemorrhagic anaemia ") that occurs following an acute loss of blood.

In view of the fact that changes in platelet numbers or shape at present add nothing to what is more easily demonstrated by other measurements, such as the erythrocyte count, it is felt that, as yet, platelets counts have little value in the clinical haematology of sheep.

PHYSICAL PROPERTIES

Table VII gives the mean figures for various physical attributes of the blood for sheep suffering from a variety of pining conditions. Using the red count as the independent variate it can be seen that the figures for sedimentation in scrapie and Johne's disease fall along the line given by the bled sheep in Table I but that in worm infestation the rate appears less rapid. With viscosity, the figures for scrapie, hypochromic anaemia, and oligocythaemia also fall along the line given by Table I, but the other conditions, including the mean for healthy sheep, give smaller viscosity figures. The figures for specific gravity give a fairly straight line when plotted against the red count, with an exception in the case of oligocythaemia.

TABLE VII
AVERAGE VALUE FOR PHYSICAL PROPERTIES IN PINING CONDITION

	Red Count	*Viscosity*	*S.G.*	*Sediment*	*Fragility*
Healthy	11·5	5·8	1,052	3	0·55
Worms*	9·2	4·2	—	1	0·50
Worms	8·7	—	1,042	—	—
Worms†	8·5	6·9	—	—	0·50
Cobalt deficient* ...	11·6	5·2	1,053	—	0·50
Solway pine* ...	10·3	5·0	1,046	—	0·55
Johne's disease ...	9·6	4·0	1,043	5	0·57
Scrapie	11·5	6·7	—	4	0·50
Oligocythaemia ...	9·0	4·9	1,055	—	0·50
Hypochromic lambs	9·2	5·3	—	—	0·50

* Stewart and Holman (1944).
† Holman and Pattison (1941) mixed infestation with *H. contortus* predominant.

Although the mean figures for these ailments show differences in their correlation with the red count, an inspection of the individual figures showed little correlation with the different diseases and offered no easy method of differential diagnosis among the different conditions. These measurements, therefore, in the present state of knowledge, merely confirm what is more accurately measured by other methods, such as the haemoglobin estimation or erythrocyte count. In view of this it does not seem worth while setting standards for these qualities or recommending that they should be used in routine examinations until further investigations have shown what factors among sheep having the same red count are responsible for the wide variation in these measurements.

The only abnormal figures for sedimentation rate were obtained from two ewes affected with gangrenous mastitis. Although the erythrocyte counts for these ewes were normal, falls of 22 and 32 mm. per 24 hours were recorded. Abnormal viscosity figures were obtained in two ewes: one, affected with pregnancy toxaemia, in which, despite a normal erythrocyte count, the observed figure was 23 and the following day 20; the other, a ewe affected with enzootic pneumonia in which a figure of 9·8 was recorded. Other ewes, affected with pneumonia, gave figures of 8·8 and 8·2 for normal red counts.

Fragility was not affected by pining conditions and a change in fragility would appear specific for haemolytic anaemia, where erythrocytes may show total haemolysis in tubes containing even 7·5 per cent. of NaCl. With fragility, however, the difficulty lies in consistently making up solutions of the exact strength with salt that is dried to the same degree, and it may well be that sheep recorded as normal with figures of as little as 0·45, or as much as 0·65 per cent., represent errors in technique. It is therefore preferable either to use healthy sheep as a control or, with experimental animals, to make use of the M.A.D. (0·04 per cent. alteration in 24 hours).

With coagulation time the wide distribution destroys the utility of the normal range and among the numerous experimental and natural cases of disease that have been examined only one case of delayed coagulation time has been observed. This was a case of a ewe severely affected with jaundice in which the coagulation time was seven minutes; in addition, there was persistent bleeding from the puncture. For comparison, a less severe case of this disease, seen in a lamb, showed a coagulation time of only two minutes. Among the experimental animals were five sheep injected with fatal doses of *Cl. welchii* type D toxin. Here, although the individual figures remained within the normal range, the decrease in coagulation time observed in the group was significant ($t = 5{\cdot}22$, $n = 4$ and $p = 0{\cdot}01/0{\cdot}001$). Here again, unless statistical tests are used, the M.A.D. of 1·5 minutes for coagulation time is the more sensitive standard for experimental animals, and with this standard two of the sheep showed a significant decrease in coagulation time with a third sheep on the border line with a decrease of 1·5 minutes.

SUMMARY

The anaemias of sheep are classified as post-haemorrhagic, haemolytic and dyshaemopoietic. Dyshaemopoietic anaemies are further classified as simple oligocythaemia, hypochromia, "hyperchromic" (normochromic macrocytic) anaemia of Innes and Shearer, and aplastic anaemia. Microcytic anaemia has not been observed and macrocytic anaemia is remarkable in that no increase in corpuscular diameter could be demonstrated.

Primary polycythaemia has not been recorded but secondary polycythaemia (erythrocytosis) was reported by Fourie, and relative

polycythaemia (anhydraemia) occurred in some cases of worm infestation and in poisoning by *Cl. welchii* type D toxin.

Standards for the classification of abnormalities in erythrocytes are given as a table.

Changes in the size and shape of thrombocytes have been recorded in haemolytic anaemia (Chamberlin) and in post-haemorrhagic anaemia. Thrombocytopenia has not been recorded. The counting of platelets by the technique employed (Holman 1944b) appears of little practical value.

Small changes in both fragility and coagulation time are more easily distinguished, where possible, by the use of the M.A.D. (Holman, 1944b) than by the use of the normal range. An increase in fragility was recorded in haemolytic anaemia due to *Cl. welchii* type A toxin, and a decrease in coagulation time was produced by lethal doses of *Cl. welchii* type D toxin.

Other physical measurements, specific gravity, sedimentation and viscosity, showed great irregularity and were of no help in a differential diagnosis among the pining conditions.

REFERENCES

Andrew, J. S. (1942). *J. agric. Res.*, **65**, 1.

Beattie, J. M., and Dickson, W. E. C. (1948). *A Textbook of Pathology.* 4th ed. London: Heinemann.

Chamberlin, W. E. (1933). *Aus. vet. J.*, **9**, 2.

Fourie, P. J. J. (1931). *Rep. Director vet. Research, Dept. Agric., Onderstep., S. Africa*, **17**, 495.

Fraser, A. C. (1930). *Rep. Inst. anim. Path., Univ. Camb.*, **1**, 114.

Gordon, W. S., Stewart, J., Holman, H. H., and Taylor, A. (1940). *J. Path. Bact.*, **50**, 251.

Groen, J. (1940). *Chem. Weekblad*, **37**, 106. Quoted from: *Biol. Abstracts* (1941), **15**, 23316.

Holman, H. H. (1944a). *J. comp. Path.*, **54**, 26; (1944b). *Ibid.*, 179; (1944c). *Ibid.*, 207.

Holman, H. H., and Pattison, I. H. (1941). *Vet. Rec.*, **53**, 491.

Innes, J. R. M., and Shearer, G. D. (1940). *J. comp. Path.*, **53**, 1.

Vaughan, J. M. (1936). *The Anaemias.* London: Oxford Univ. Press.

Whitby, L. E. H., and Britton, C. J. C. (1939). *Disorders of the Blood.* 3rd ed. London: Churchill.

Wintrobe, M. M. (1942). *Clinical Haematology.* London: Kempton.

Wirth, D. (1931). *Grundlagen einer Klinischen Hämatologie der Haustiere.* Berlin: Urban and Schwarzenberg.

Wirth, D. (1938). *13th Inter. vet. Congress.* p. 273.

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* *Meyer, E., and Arnold, L., Amer. J. Digest. Dis., 5:418*

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